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(54) Title: COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CONDITIONS, DISORDERS, OR DISEASES INVOLVING CELL DEATH

(57) Abstract:

# PATENT COOPERATION TREATY

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## DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter* and 39)

| | | |
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| Applicant's or agent's file reference 10001-006-228 | IMPORTANT DECLARATION | Date of mailing *(day/month/year)* 17 JUL 2001 |
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| Applicant COGENT NEUROSCIENCE, INC. | | |

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories.
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☐ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practiced on the human or animal body.
   - l. ☐ mere presentations of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.

2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   ☒ the description ☒ the claims ☒ the drawings

3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out.
   - ☒ the written form has not been furnished or does not comply with the standard.
   - ☐ the computer readable form has not been furnished or does not comply with the standard.

4. Further comments:

Please See Continuation Sheet.

| | |
|---|---|
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | Authorized officer MARY TUNG (703) 308-0196 |

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4. Further Comments (Continued):

A meaningful search cannot be performed on the instant claims.
The claims appear to encompass an unfathomable number of inventions because of the number of claimed sequences.

It is noted that the instant claim set is apparently drawn to a multitude of DNA or amino acid sequences shown in Figures 4-13. Each of Figures 4-13 are apparently broken up into a multitude of subfigures. Claim 1 (a) apparently refers to "an amino acid sequence which is shown in all of Figures 4A-AB". It is not at all clear how one sequence can be the same as all those separate, distinct sequences. Perhaps applicant intended to refer to the various subfigures in the alternative? Or maybe the open claim language "comprising" as in am amino acid sequence which comprises all of those shown in Figures 4A-AB.

Figures 4-13 are defective because the sheets are not numbered in consecutive Arabic numbers. See PCT/RO/106 mailed May 4, 2001. Further, the first sheet of each of the Figures 4-13 contains a heading "open reading frame for..." and the text "Fig No." These words are missing from the subsequent sheets of each of the subfigures 4-13. The figures are not clearly labeled. Further, wtih regard to the headings, PCT Rule 11.11(a) prohibits words in the drawings.

Each figure does not have a uniques label which says "Fig. No. 4A, Figure No. 4B", etc. Some of the figures apparently contain subfigures which run over onto the next sheet. See 10L, 10J,etc.Further, some of the figures apprently contain subfigures which so not have a figure label, see the text in the box above Figure 10P. Furthermore, the numbering system of the sequences appears to be incorrect, see numbers 340 and 341, both denoting the same position of the last line of amino acids of Figure 10H. None of the abberations are permissible and a search of any such material would not be meaningful.

Beyond all these errors, the overriding problem with performing a search on the claims is that in the figures and of the Brief Description of the Figures, pages 13-15, no SEQ ID Nos are provided. Without any such correlation, it is impossible to determine which of te sequences recited int he claims correspond to those recited in the sequence listing. Absent that correlation, it is impossible to determine the full scope of the claimed invention or to search even a portion of the claimed invention.

PCT Rule 6.2(a) states that the claims shall not, except when absolutely necessary, rely, in respect to the technical features of the invention, on references to the description of the drawings. In particular, they shall not rely on such references as "as described in part...of the description" or "as illustrated in figure...of the drawings". The nature of the instant invention does not meet the criteria of "absolutely necessary" because these claims could have easily been drafted to include the particular SEQ ID Nos of the sequences. That may result in theclaim becoming rather lengthy, but this is to be expected when one claim appears to encompass hundreds of inventions.

The Authorized Officer contacted the applicants on 16 May 2001 concerning a potential Lack of Unity. However, upon further consideration, the claims have been determined to be unsearchable.

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WO 01/076532 A2

(54) Title: COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CONDITIONS, DISORDERS, OR DISEASES INVOLVING CELL DEATH

(57) Abstract:

# COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CONDITIONS, DISORDERS, OR DISEASES INVOLVING CELL DEATH

## 1 INTRODUCTION

The present invention relates to compositions and methods for the treatment and diagnosis of conditions, disorders, or diseases involving cell death, including, but not limited to, neurological disorders such as stroke. Nucleic acids are described herein which, when introduced into a cell either predisposed to undergo cell death or in the process of undergoing cell death, prevent, delay, or rescue the cell from death relative to a corresponding cell into which no exogenous nucleic acids have been introduced. Such nucleic acids are referred to as "protective sequences". Protective sequences or their products are identified by their ability to prevent, delay, or rescue a cell, cells, tissues, organs, or organisms from dying. Protective sequences or their products are also identified via their ability to interact with other genes or gene products involved in conditions or disorders involving cell death.

The invention further includes recombinant DNA molecules and cloning vectors comprising protective sequences, and host cells and host organisms engineered to contain such DNA molecules and cloning vectors. The present invention further relates to protective sequence products and to antibodies directed against such protective sequence products.

The protective sequences identified, their products, or antibodies may be used diagnostically, prophylactically, therapeutically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and prophylactic or therapeutic use of compounds in the treatment and diagnosis of conditions, disorders, or diseases involving cell death. Additionally, methods are provided for the diagnostic monitoring of patients undergoing clinical evaluation for the treatment of conditions or disorders involving cell death, for monitoring the efficacy of compounds in clinical trials and for identifying subjects who may be predisposed to such conditions, disorders, or diseases involving cell death.

## 2 BACKGROUND OF THE INVENTION

### 2.1 Mechanisms which Lead to Cell Death

It is widely recognized that at least two distinct cell death mechanisms exist for mammalian cells. These two mechanisms are necrosis and apoptosis, and are significant components of numerous conditions, disorders and disease states.

Necrosis plays an important physiologic role in signaling the presence of certain conditions. When cells die as a result of necrosis, the dying cells release substances that activate the body's immune response in a local, and in some cases widespread, reaction to the necrosis-inducing condition. This response is important in, for example, bacterial infection.

Experimental evidence in a wide variety of cells throughout the body has revealed that every cell can initiate a program of self-destruction, called apoptosis. This program can be initiated by a wide variety of natural and unnatural events. There are at least four distinct pathways for executing this program of cell death, and it is virtually certain that dozens, if not hundreds, of different intracellular biochemical cascades interact with each pathway. It is equally likely that certain cell types, such as cells in the heart or neurons, will utilize specialized signaling pathways that are not generally represented elsewhere in the body. However, since cell death is neither always necessary nor desired, it would be desirable to manipulate the manner in which cells start their death process. In some circumstances, preventing, delaying, or rescuing cells from death would either alleviate the disease or allow more time for definitive treatment to be administered to the patient. An example of this situation is brain cell death caused by ischemic stroke: preventing, delaying, or rescuing cells from death until the blood supply to the brain could be restored would greatly reduce, if not eliminate, the possibility of a person's death and/or long-term disability from stroke (Lee JM, et al. *Nature* 1999, 399(supp): A7-A14; Tarkowski E, et al. *Stroke* 1999, 30(2): 321-7; Pulera MR, et al. *Stroke* 1998, 29(12): 2622-30). In still other circumstances, the failure of cells to die may itself lead to disease such as cancer (Hetts SW. *JAMA* 1998, 297(4): 300-7).

Cell death plays an important role in the normal function of mammalian organisms. While it may seem counterintuitive for cells to have death as a normal part of their life cycle, controlled and physiologically appropriate cell death is important in regulating both the absolute and relative numbers of cells of a specific type. (Hetts SW. *JAMA* 1998, 297(4):

300-7; Garcia I, et al. *Science* 1992, 258(5080): 302-4). When the mechanism of apoptosis does not function properly and normal cell death does not occur, the resulting disease is characterized by unregulated cellular proliferation, as occurs in a neoplastic disease or an autoimmune disease (Hetts SW. *JAMA* 1998, 297(4): 300-7; Yachida M, et al. *Clin Exp Immunol* 1999, 116(1): 140-5).

One method for regulating cell death involves manipulating the threshold at which the process of cell death begins. This threshold varies significantly by cell type, tissue type, the type of injury or insult suffered by the cell, cellular maturity, and the physiologic conditions in the cell's environment (Steller H., *Science* 1995, 267(5203): 1445-9). Although it is probable that certain cellular injuries or insults irrevocably induce death, lesser injuries or insults may begin the dying process without inducing irreversible cell death. What constitutes a lesser injury or insult may vary tremendously with changes in the factors influencing that cell's death threshold. The ability to alter a cell's threshold for responding to an injury or insult, that is, to either promote or discourage cell death, would be a desirable goal for the treatment of conditions involving cell death. The ability to better control cell death, by either discouraging or promoting the mechanisms of cell death, would be an important invention for ameliorating disease (US Patents 5,925,640; 5,786,173; 5,858,715; 5,856,171).

Recent evidence suggests that the mechanisms of cellular death may be more complex than the two discrete pathways of apoptosis and necrosis. Examples of this evidence may be found in the central nervous system (CNS). In the complex CNS cellular environment, both necrosis and apoptosis are observed with commonly studied conditions, disorders, or diseases such as focal ischemia, global ischemia, toxic insults, prolonged seizures, excitotoxicity, and traumatic brain injury. In some reports, both apoptosis and necrosis have been described (Choi WS, et al. *J Neurosci Res* 1999, 57(1): 86-94; Li Y, et al. *J Neurol Sci* 1998, 156(2): 119-32; Lee J-M, et al. *Nature* 1999, 399(supp): A8-A14; Baumgartner WA, et al. *Ann Thorac Surg* 1999, 67(6): 1871-3; Fujikawa DG, et al. *Eur J Neurosci* 1999, 11(5): 1605-14; Gwag BJ, et al *Neuroscience* 1999, 90(4): 1339-48; Mitchell IJ, et al. 1998, 84(2): 489-501; Nakashima K, et al. *J Neurotrauma* 1999, 16(2): 143-51; Ginsburg, MD *Cerebrovascular Disease: Pathophysiology, Diagnosis, and Management* 1998 Ch 42; Rink AD, et al. *Soc Neurosci Abstr* 1994, 20:250(Abstract)). Similar observations also occurred with brain tumor cells. (Maurer BJ, et al. *J Natl Cancer Inst* 1999, 91(13): 1138-46)

Other investigators found that neurons die by either apoptosis or necrosis under different environmental conditions (Taylor DL, et al. *Brain Pathol* 1999, 9(1): 93-117). There also are reports of a unique type of neuronal cell death following stroke. This new type of cell death has features common to both necrosis and apoptosis (Fukuda T, et al. *Neurosci Res* 1999, 33(1): 49-55). Other investigators believe that neuronal cell death is best represented by a continuum between apoptosis and necrosis, possibly mediated by calcium levels (Lee J-M, et al.1999, 399(supp): A7-A14), or a combination of direct ischemic damage followed by indirect damage from excitotoxicity and loss of interneuronal connections (Martin LJ, et al. *Brian Res Bull* 1998, 46(4): 281-309). Further complicating the picture of neuronal cell death is the observation that the death of one or more neurons in one region of the brain can induce the death of neurons in other brain regions. This phenomenon has been observed with stroke as described above (Martin LJ, et al. *Brain Res Bull* 1998, 46(4): 281-309) as well as neuronal cell death induced by the withdrawal of growth factors (Ryu BR, et al. *J Neurobiol* 1999, 39(4): 536-46). Given the complex nature of actions and interactions among the many physiologic and molecular forces in brain tissue, and the different abilities of many substances acting either alone or in combination to induce cellular injury or death, it is difficult to determine with any degree of certainty if a nerve cell death process is due to apoptosis or necrosis (Graham DI, *Greenfield's Neuropathology* Ch 3 1997).

Despite the challenges in classifying the mechanism of cellular death, there is broad agreement that most, if not all, cells share common features in their death mechanisms (see, *e.g.*, Lee J.M., et al., *Nature* 1999, 399 (supp): A7-A14).

### 2.2 Selected Factors and Conditions which Inhibit Cell Death Mechanisms

Several factors have been reported to inhibit the cell death pathway. One of the best-known factors is the gene product *bcl-2* (Adams JM, et al. *Science* 1998, 281(5381): 1322-6; Vaux DL, et al. *Proc Natl Acad Sci* 1993, 90(3): 786-9; US Patent 5,856,171 and references cited therein). Expression of *bcl-2* is believed to regulate apoptotic death in neurons, kidney, heart, liver, blood and skin cells under experimental conditions. In addition to regulating death by apoptosis, *bcl-2* is believed to regulate death caused by non-apoptotic mechanisms. Factors related to *bcl-2* have been shown to be over-expressed in cancer and autoimmune conditions, disorders, or diseases (US Patent 5,856,171 and references cited

therein). Other related factors acting on the same pathway as *bcl-2* also delay or prevent cell death.

In the brain, several factors have been shown to influence the cell death pathway. In excitotoxic injury to neurons, it was shown that lithium or *bcl-2* each individually protected neurons against cell death (Nonaka S, et al. *Proc Natl Acad Sci* 1998, 95(5): 2642-7; Behl C, et al. *Biochem Biophys Res Commun* 1993, 197(2): 949-56). During ischemic injury to neurons, it was shown that nerve growth factor (NGF) and *bcl-2* individually offered protection against neuronal death (Guegan C, et al. *Neurobiol Dis* 1999, 6(3): 180-9; Linnik MD, et al. *Stroke* 1995, 26(9): 1670-4).

Factors acting to prevent cell death do not act solely in the brain. In the heart, increased tolerance to non-lethal ischemic injury was associated with an increased expression of the *bcl-2* gene, suggesting that *bcl-2* was involved in protecting the cardiac muscle cells against ischemic injury (Maulik N, et al. *Ann NY Acad Sci* 1999, 874:401-11). This same study demonstrated that lower levels of *bcl-2* expression were associated with higher rates of cardiac cell death. A similar result was found for mechanical injury to heart papillary muscle cells.

Recently, it has been demonstrated that *bcl-2* prevented cell death in a brain ischemia model (Guegan C, et al. *Neurobiol Dis* 1999, 6(3): 180-9; Linnik MD, et al. *Stroke* 1995, 26(9): 1670-4). It was shown that the activity of *bcl-2* to prevent neuronal death was consistently demonstrated across several different physiologic insults. It also has been demonstrated that the distinction between apoptotic death and necrotic death is open to question, so the possibility exists that *bcl-2* can prevent or delay the necrotic cell death pathway, the apoptotic cell death pathway or perhaps an as yet undemonstrated cell death pathway.

Preventing cell death is an important medical goal. Several types of mammalian cells, most notably neurons and cardiac muscle cells, have limited if any capacity to regenerate. Preventing the death of these cells from conditions such as heart attack, stroke, shock, infection, cancer, Alzheimer's disease or traumatic injury, to name a few, would be an important medical advance as the heart and brain cannot grow sufficient cells to replace those cells lost to disease or infection.

In addition to preventing cell death, delaying and/or rescuing cells from programmed cell death is also an important medical goal. In many pathological conditions where there is an expectation that the disease will be successfully treated, such as many types of infection, hypoxia, ischemia or metabolic disturbances, delaying cell death would allow the pathological condition to be treated without permanent damage to the cells. In other words, the cells may be put into a suspended state from which they could successfully be rescued and emerge with their normal function intact.

## 3 SUMMARY OF THE INVENTION

The present invention relates to the discovery, identification and characterization of protective sequences and to compositions and methods for the treatment and diagnosis of conditions, disorders, or diseases involving cell death. Protective sequences refer to nucleic acid molecules comprising nucleic acid sequences which, when introduced into a cell either predisposed to undergo cell death or in the process of undergoing cell death, prevent, delay, or rescue the cell from death relative to a corresponding cell into which no exogenous nucleic acids have been introduced. For example, protective sequences may act to prevent, delay, ameliorate, inhibit, reduce, or rescue neuronal cell death (*e.g.* apoptosis, necrosis and related cellular events). The invention further relates to the discovery, identification and characterization of gene products encoded by such nucleic acid molecules, or by degenerate, *e.g.*, allelic or homologous, variants thereof. Protective sequences also can be regulatory nucleic acids. Protective sequences further can be both coding sequences and regulatory sequences.

The invention further relates to target sequences. Target sequences include, but are not limited to, upstream and downstream regulatory sequences, upstream and downstream complete or partial gene or gene product sequences, antibodies, antisense molecules or sequences, ribozyme molecules, and other inhibitors or modulators directed against such protective sequences and protective sequence products.

Protective sequences and protective sequence products can be utilized prophylactically and/or therapeutically to prevent, delay ameliorate, inhibit, reduce, or rescue conditions of cell death or symptoms of conditions, disorders, or diseases involving cell death. The modulation of the expression of protective sequences, *e.g.*, endogenous protective

sequences, and/or the activity of the protective sequence products, *e.g.*, endogenous protective sequence products, can also be utilized prophylactically or therapeutically to prevent, delay, ameliorate, inhibit, reduce, or rescue conditions of cell death or symptoms of conditions, disorders, or diseases involving cell death. Further, protective sequences and protective sequence products can be used to diagnose individuals exhibiting or predisposed to such conditions, disorders, or diseases involving cell death.

The compositions of the present invention include, in particular, nucleic acid molecules which comprise the following sequences: (a) nucleic acids of protective sequences, as well as allelic variants, homologs, mutants and fragments thereof; (b) nucleic acids which encode protective sequence products; (c) nucleic acids which encode protective sequence regulatory elements; (d) nucleic acids which encode fusion proteins comprising protective sequence products or one or more protective sequence product domains fused to a heterologous polypeptide; (e) nucleic acids which encode fusion proteins comprising protective sequence regulatory elements fused to a heterologous polypeptide; (f) nucleic acids which hybridize to the above described sequences under highly stringent or moderately stringent conditions, including, but not limited to, human homologs; and (g) complementary (*e.g.*, antisense) nucleic acids of the sequences described in (a) through (f), above. The nucleic acid molecules of the invention include, but are not limited to, cDNA, genomic DNA (including non-expressed features such as introns) and RNA sequences.

The present invention also encompasses expression gene products of the protective sequences listed above; *i.e.*, proteins and/or polypeptides that are encoded by the above protective sequences. The present invention also encompasses expression gene products generated by differentially or alternately splicing the protective sequences listed above. Nucleic acid molecules that can separately encode these differentially or alternatively spliced gene products are also included in the invention.

Mimics, agonists and antagonists of the protective sequences, protective sequence products, genes, gene products, or their regulatory elements are also included in the present invention. Such mimics, agonists and antagonists will include, for example, small molecules, large molecules (*e.g.*, protective sequence product fragments or protective sequence product ligands) and antibodies directed against a protective sequence product. Mimics, agonists and antagonists of the invention also include nucleic acids, such as antisense

and ribozyme molecules, and gene or regulatory sequence replacement constructs, which can be used to modulate, inhibit or enhance expression of a protective sequence.

The present invention further encompasses cloning and expression vectors, which may include, but are not limited to, bacterial, fungal, insect, plant, and mammalian vectors, which contain the protective nucleic acid sequences of the invention, which can be used as probes or to express those protective nucleic acid sequences, protective sequence products, genes and/or gene products in host cells or organisms. The present invention also relates to cells that have been transformed, transfected, or infected with such vectors, and to cells engineered to contain or express the protective nucleic acid sequences, protective sequence products, genes, gene products, and/or regulatory elements of the invention. Further, non-human host organisms which have been transformed, transfected, or infected with these protective nucleic acid sequences, or their regulatory elements, are also encompassed in the present invention. Host organisms of the invention include organisms transformed, transfected, or infected with the cloning vectors described above, including, but not limited to, non-human transgenic animals, and particularly transgenic non-human mammals which have been engineered to express a protective sequence, protective sequence product, gene, gene product, or regulatory element of the invention, or "knock-outs" which have been engineered to not express the protective sequence, protective sequence product, gene, gene product, or regulatory element of the invention.

The transgenic animals of the invention include animals which express a mutant variant or polymorphism of a protective sequence, protective sequence product, gene, gene product, or regulatory element, particularly a mutant variant or polymorphism of a protective sequence, protective sequence product, gene, gene product, or regulatory element which is associated with a condition, disorder, or disease involving cell death. The transgenic animals of the invention further include those that express a protective sequence transgene at higher or lower levels than normal.. The transgenic animals of the invention further include those which express the protective sequence, protective sequence product, gene, gene product, or regulatory element in all their cells, "mosaic" animals which express the protective sequence, protective sequence product, gene, gene product, or regulatory element in only some of their cells, and those in which the protective sequence, protective sequence product, gene, gene product, or regulatory element is selectively introduced into and expressed in a specific

cell type(s). The transgenic animals of the invention also include "knock-out" animals. Knock-out animals comprise animals that have been engineered to no longer express the protective sequence, protective sequence product, gene, gene product, or regulatory element.

The present invention also relates to methods and compositions for the diagnosis of conditions, disorders, or diseases involving cell death, as well as for the identification of subjects susceptible to such conditions, disorders, or diseases. Such methods comprise, for example, measuring expression of the protective sequence, protective sequence product, gene, gene product, or regulatory element in a patient sample, or detecting a mutation in the protective sequence, protective sequence product, gene, gene product, or regulatory element in the genome of a mammal, including a human, suspected of exhibiting such a condition, disorder, or disease. The protective nucleic acid molecules of the invention can be used also as diagnostic hybridization probes, or as primers for diagnostic PCR analysis to identify protective sequences, protective sequence products, genes, gene products, or regulatory element mutations, allelic variations or regulatory defects, such as defects in the expression of the protective sequence, protective sequence product, gene, gene product, or regulatory element. Such diagnostic PCR analyses can be used to diagnose individuals with a condition, disorder, or disease involving cell death associated with a particular protective sequence, protective sequence product, gene, gene product, or regulatory element mutation, allelic variation or regulatory defect. Such diagnostic PCR analyses can be used also to identify individuals susceptible to such conditions, disorders, or diseases involving cell death.

Methods and compositions, including pharmaceutical compositions, for the treatment of conditions, disorders, or diseases involving cell death also are included in the invention. Such methods and compositions can increase, decrease or otherwise modulate the level of protective sequences, protective sequence products, genes, gene products, or their regulatory elements in a patient in need of such treatment. Such methods and compositions can also modulate the level of protective sequence expression (*e.g.*, endogenous protective sequence expression) and/or the level of activity of a protective sequence product, (*e.g.*, endogenous protective sequence product). Further, since the protective sequence or protective sequence product need not normally be involved in such conditions, disorders, or diseases, such methods include, for example, modulating the expression of the protective sequence and/or the activity of the protective sequence product for the treatment of conditions,

disorders, or diseases involving cell death which are normally mediated by some other gene.

In one embodiment, such methods and compositions are utilized for the treatment of the types of conditions, disorders, or diseases, which can be prevented, delayed or rescued from cell death and include, but are not limited to, those associated with the central nervous system including neurological and psychiatric conditions, disorders, or diseases; those of the peripheral nervous system; conditions, disorders, or diseases caused by physical injury; conditions, disorders, or diseases of the blood vessels or heart; conditions, disorders, or diseases of the respiratory system; neoplastic conditions, disorders, or diseases; conditions, disorders, or diseases of blood cells; conditions, disorders, or diseases of the gastrointestinal tract; conditions, disorders, or diseases of the liver; conditions, disorders, or diseases of the pancreas; conditions, disorders, or diseases of the kidney; conditions, disorders, or diseases of the ureters, urethra or bladder; conditions, disorders, or diseases of the male genital system; conditions, disorders, or diseases of the female genital tract; conditions, disorders, or diseases of the breast; conditions, disorders, or diseases of the endocrine system; conditions, disorders, or diseases of the thymus or pineal gland; conditions, disorders, or diseases of the skin or mucosa; conditions, disorders, or diseases of the musculoskeletal system; conditions, disorders, or diseases causing a fluid or hemodynamic derangement; inherited conditions, disorders, or diseases; conditions, disorders, or diseases of the immune system or spleen; conditions, disorders, or diseases caused by a nutritional disease; and conditions, disorders, or diseases typically occurring in infancy or childhood, as described in Section 5.4.1.1. below.

In yet another embodiment, the methods and compositions of the invention are utilized for the prevention, or delay, of cell death in the event of one or more infections which may be caused by bacteria; viruses; members of the family rickettsiae or chlamydia; fungi, yeast, hyphae or pseudohyphae; prions; protozoans; or metazoans.

In a further embodiment, the compounds and methods of the invention can be used to treat infections or conditions, disorders, or diseases which cause cell death in organ systems including, but not limited to, blood vessels, heart, red blood cells, white blood cells, lymph nodes, spleen, respiratory system, oral cavity, gastrointestinal tract, liver and biliary tract, pancreas, kidney, lower urinary tract, upper urinary tract and bladder, male sexual organs and genitalia, female sexual organs and genitalia, breast, thyroid gland, adrenal gland, parathyroid gland, skin, musculoskeletal system, bone marrow or bones.

In another embodiment, the compounds and methods of the invention can be used to treat further physiological impacts on organs caused by the infections which induce cell death including, but not limited to, fever equal to or greater than 101.5 degrees Fahrenheit, a decrease or increase in pulse rate by more than 20 beats per minute, a decrease or increase in supine systolic blood pressure by more than 30 millimeters of mercury, an increase or decrease in respiratory rate by more than 8 breaths per minute, an increase or decrease in blood pH by more than 0.10 pH units, an increase or decrease in one or more serum electrolytes outside of the clinical laboratory's usual reference range, an increase or decrease in the partial pressure of arterial oxygen or carbon dioxide outside of the clinical laboratory's usual reference range, an increase or decrease in white or red blood cells outside of the laboratory's usual reference range, an acute confusional state such as delirium where delirium is defined by the American Psychiatric Association's DSM-IV Manual or a diminished level of consciousness or attention.

In another embodiment, the compounds and methods of the invention can be used to promote cell death. These compounds could be useful for treating and/or ameliorating conditions caused by, for example, cancer and autoimmune diseases, both of which are manifested by an uncontrolled growth of cells.

The invention still further relates to methods for identifying compounds which modulate the expression of a protective sequence and/or the synthesis or activity of a protective sequence product. Such compounds include therapeutic compounds which can be used as pharmaceutical compositions to reduce or eliminate the symptoms of conditions, disorders, or diseases involving cell death. Cellular and non-cellular assays are described which can be used to identify compounds which interact with a protective sequence, protective sequence product, gene, gene product, and/or regulatory element, *e.g.*, modulate the activity of a protective sequence and/or bind to a protective sequence product. Such cell-based assays of the invention utilize cells, cell lines, or engineered cells or cell lines that express the protective sequence, protective sequence product, gene, gene product, and/or regulatory element.

In one embodiment, such methods comprise contacting a compound to a cell which expresses a protective sequence, protective sequence product, gene, gene product, and/or regulatory element, measuring the level of protective sequence expression, gene

product expression or gene product activity, and comparing this level to the level of protective sequence expression, gene product expression or gene product activity produced by the cell in the absence of the compound, such that if the level obtained in the presence of the compound differs from that obtained in its absence, a compound which modulates the expression of the protective sequence and/or the synthesis or activity of protective sequence products has been identified.

In an alternative embodiment, such methods comprise administering a compound to a host, *e.g.*, a transgenic animal which expresses a protective sequence transgene or a mutant protective sequence transgene, and measuring the level of protective sequence expression, gene product expression or gene product activity. The measured level is compared to the level of protective sequence expression, gene product expression or gene product activity in a host which is not exposed to the compound, such that if the level obtained when the host is exposed to the compound differs from that obtained when the host is not exposed to the compound, a compound which modulates the expression of the protective sequence and/or the synthesis or activity of protective sequence products, and/or the symptoms of conditions, disorders, or diseases involving cell death, has been identified.

### 3.1 Definitions

"Protective sequence", as used herein, refers to nucleic acid molecules comprising nucleic acid sequences which, when introduced into a cell predisposed to either undergo cell death or in the process of undergoing cell death, prevent, delay, or rescue the cell from death relative to a corresponding cell into which no exogenous protective nucleic acids have been introduced. In one embodiment, a protective sequence encodes a protective sequence product. In another embodiment, protective sequences are any transcriptional products of the sequences disclosed herein. In another embodiment, protective sequences comprise regulatory elements of the sequences disclosed herein which modulate the expression of a nucleic acid within a cell. For example, protective sequences, their products, or their regulatory elements may act to prevent, delay, or rescue a cell, cells, tissues, organs, or organisms from dying. Compounds which modulate protective sequence expression or activity of the protective sequence product can be used in the treatment of conditions, disorders or diseases associated with cell death processes. It is to be understood that the

protective sequences described above can act to ameliorate or delay symptoms related to cell death. Although the protective sequences may be involved directly in such cell death related conditions or disorders, in certain cases, the protective sequences will not normally be involved in such conditions or disorders, but will be effective for the treatment and/or prevention of such disorders. In these cases, modulation of the expression of the protective sequence and/or the activity of the protective sequence product will be useful for the treatment of conditions, disorders, or diseases involving cell death which are normally mediated by some other gene.

"Cell death", as used herein, refers to any mechanism and/or pathway whereby a cell undergoes a series of events which ultimately would lead to the death of the cell. For example, cell death may be caused by various processes including, but not limited to, apoptosis or programmed cell death, necrosis, or an as yet unidentified cell death pathway. Cell death may be induced in individual cells as a consequence of numerous internal and external stimuli including, but not limited to, genetic predisposition, toxic chemicals or processes, heat, cold, rapid environmental changes, radiation, viruses, prions, bacteria, disruption of nutrient balance, or exposure to bi-products and signaling from other cells undergoing cell death. The protective sequences disclosed herein, when introduced into a cell (*e.g.* a neuronal cell) which has undergone an event that would ultimately lead to cell death (*e.g.* ischemia), are capable of rescuing the cell from cell death. Moreover, when a protective sequence, in combination with a reporter gene (*e.g.* green fluorescent protein), is introduced into a cell which has undergone an event that would ultimately lead to cell death, expression of the reporter gene is an indication that the protective sequence is capable of rescuing the cell from cell death.

## 4 BRIEF DESCRIPTION OF THE FIGURES

Figures 1(A-J). Protective nucleic acids. See Table 1 for the identity, the sequence identifier number, the length in base pairs and the Accession Number for each of the sequences shown in these figures.

Figure 2. Restriction map and diagram of plasmid pCMV·SPORT2. This plasmid was used as the cloning vector for the protective sequences. Each clone was ligated

into the *Sal*I-*Not*I restriction sites of the plasmid.

Figures 3(A- F). Protected Cortical Neurons Visualized by Detection of EGFP Expressing Cells. Figures 3A and 3B represent non-stroked, positive control samples. Figure 3C represents a positive control, stroked sample using Bcl-2. Figure 3D represents a stroked, negative control sample. Figure 3E represents a stroked sample protected by a representative protective sequence. Figure 3F presents the average number of neurons that survived for three days in both a stroked sample protected by a protective sequence and a corresponding stroked, negative control sample.

Figures 4(A-AB). Open Reading Frames for CNI-00718. This Figure depicts the 28 potential ORFs for CNI-00718. Also shown are the nucleotide sequences which encode the ORFs.

Figures 5(A-L). Open Reading Frames for CNI-00722. This Figure depicts the 12 potential ORFs for CNI-00722. Also shown are the nucleotide sequences which encode the ORFs.

Figures 6(A-K). Open Reading Frames for CNI-00725. This Figure depicts the 11 potential ORFs for CNI-00725. Also shown are the nucleotide sequences which encode the ORFs.

Figures 7(A-Z). Open Reading Frames for CNI-00726. This Figure depicts the 26 potential ORFs for CNI-00726. Also shown are the nucleotide sequences which encode the ORFs.

Figures 8(A-S). Open Reading Frames for CNI-00727. This Figure depicts the 19 potential ORFs for CNI-00727. Also shown are the nucleotide sequences which encode the ORFs.

Figures 9(A-X). Open Reading Frames for CNI-00728. This Figure depicts the 24 potential ORFs for CNI-00728. Also shown are the nucleotide sequences which encode the ORFs.

Figures 10(A-V). Open Reading Frames for CNI-00729. This Figure depicts the 22 potential ORFs for CNI-00729. Also shown are the nucleotide sequences which encode the ORFs.

Figures 11(A-I). Open Reading Frames for CNI-00730. This Figure depicts the 9 potential ORFs for CNI-00739. Also shown are the nucleotide sequences which encode the ORFs.

Figures 12(A-G). Open Reading Frames for CNI-00731. This Figure depicts the 7 potential ORFs for CNI-00731. Also shown are the nucleotide sequences which encode the ORFs.

Figures 13(A-H). Open Reading Framès for CNI-00732. This Figure depicts the 8 potential ORFs for CNI-00732. Also shown are the nucleotide sequences which encodes the ORFs.

## 5 DETAILED DESCRIPTION OF THE INVENTION

Protective sequences of the invention are described herein. Also described are recombinant, cloned and degenerate variants, homologs, orthologs, mutants and fragments thereof. The compositions of the invention further include protective sequence products (*e.g.* proteins or RNA) which are encoded or produced by the nucleic acid molecules of the invention, and the modulation of protective sequence expression and/or gene product activity in the treatment of conditions, disorders, or diseases involving cell death. Further, antibodies directed against the protective sequence products, or conserved variants or fragments thereof, and viral-, cell-, plant-, and animal-based models by which the protective sequences may be further characterized and utilized are also discussed in this section.

### 5.1 The Protective Sequences

The protective sequences of the invention are described in this section. Specifically, these protective sequences have been shown to prevent, delay, or rescue cell death in a cell predisposed for undergoing cell death, whether the pathway that leads to the cell death involves apoptosis, necrosis or an as yet undefined pathway. The protective sequences, their SEQ ID NOS and additional information related to the protective sequences are listed below, in Table 1.

The protective sequences listed in Table 1 may be obtained using cloning methods well known to those skilled in the art, including but not limited to the use of appropriate probes to detect the protective sequences within an appropriate cDNA or gDNA (genomic DNA) library. (See, for example, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, which is incorporated by reference herein in its entirety). Probes for the novel sequences reported herein may be obtained directly from CNI-NPP2-CP10, which represents a composite deposit containing the isolated clones, which was deposited with the ATCC as Accession No. PTA-1492 on March 16, 2000. Alternatively, oligonucleotide probes for the novel protective sequences may be synthesized based on the DNA sequences disclosed herein.

**TABLE 1**

**PROTECTIVE SEQUENCES**

| Protective sequence | SEQ ID NO: | Figure No. | Length (bp) (NotI-SalI fragment) |
|---|---|---|---|
| *CNI-00718* | *1* | *1A* | *1794* |
| *CNI-00722* | *58* | *1B* | *810* |
| *CNI-00725* | *83* | *1C* | *920* |
| *CNI-00726* | *106* | *1D* | *2144* |
| *CNI-00727* | *159* | *1E* | *1293* |
| *CNI-00728* | *198* | *1F* | *1466* |
| *CNI-00729* | *247* | *1G* | *1659* |
| *CNI-00730* | *292* | *1H* | *722* |
| *CNI-00731* | *311* | *1I* | *364* |
| *CNI-00732* | *326* | *1J* | *1046* |

The isolated protective nucleic acid molecules of the invention include, in particular, nucleic acid molecules which comprise the following sequences: (a) nucleic acids of protective sequences, as well as allelic variants, homologs, mutants and fragments thereof; (b) nucleic acids which encode protective sequence products and/or their regulatory elements, or fragments thereof; (c) nucleic acids which encode fusion proteins comprising protective sequence products and/or their regulatory elements, or one or more protective sequence product domains and/or their regulatory elements fused to a heterologous polypeptide; (d) nucleic acids which hybridize to the above described sequences under highly stringent or moderately stringent conditions, including, but not limited to human homologs; and (e) complementary (*e.g.*, antisense) nucleic acids of the sequences described in (a) through (d), above. The nucleic acid molecules of the invention include, but are not limited to, cDNA, genomic DNA and RNA sequences.

The nucleic acids of the invention also include nucleic acids which have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more nucleic acid identity to the protective nucleic acids of (a)-(d) above. The nucleic acids of the invention further include nucleic acids which encode polypeptides having at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or higher amino acid sequence identity to the polypeptides encoded by the protective nucleic acids of (a)-(d).

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical overlapping positions/total # of positions x 100). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences also can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a

mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA 87*:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA 90*:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.* (1990) *J. Mol. Biol. 215*:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleic acids homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.25*: 3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS 4*:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

The nucleic acids of the invention further include: (a) any nucleic acid which hybridizes to a nucleic acid molecule of the invention under moderately stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C, or (b) under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C, or under other hybridization conditions which are apparent to those of skill in the art (*see*, for example, Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at pp. 6.3.1-6.3.6 and

2.10.3). Preferably the nucleic acid molecule that hybridizes to the nucleic acid of (a) and (b), above, is one which comprises the complement of a nucleic acid molecule which encodes a protective sequence product. In a preferred embodiment, nucleic acid molecules comprising the nucleic acids of (a) and (b), above, encode protective sequence products.

Functionally equivalent protective sequence products include naturally occurring protective sequence products present in the same or different species. Functionally equivalent protective sequence products also include gene products which retain at least one of the biological activities of the protective sequence products, and/or which are recognized by and bind to antibodies (polyclonal or monoclonal) directed against the protective sequence products.

Among the nucleic acid molecules of the invention are deoxyoligonucleotides ("oligos") which hybridize under highly stringent or moderately stringent conditions to the nucleic acid molecules described above. In general, for probes between 14 and 70 nucleotides in length the melting temperature (TM) is calculated using the formula: Tm (°C)=81.5+16.6(log[monovalent cations (molar)])+0.41 (% G+C)-(500/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature is calculated using the equation Tm (°C)=81.5+16.6(log[monovalent cations (molar)])+0.41(% G+C)-(0.61% formamide)-(500/N) where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below Tm (for DNA-DNA hybrids) or 10-15 degrees below Tm (for RNA-DNA hybrids).

Exemplary highly stringent conditions may refer, *e.g.*, to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for about 14-base oligos), 48°C (for about 17-base oligos), 55°C (for about 20-base oligos) and 60°C (for about 23-base oligos).

Fragments of the nucleic acid molecules can be at least 9 nucleotides in length. Fragments of the nucleic acid molecules can refer also to exons or introns, and, further, can refer to portions of coding regions that encode domains of protective sequence products.

The invention also encompasses (a) DNA vectors which contain any of the foregoing coding sequences and/or their complements (*i.e.*, antisense); (b) DNA expression vectors which contain any of the foregoing coding sequences operatively associated with a regulatory element which directs the expression of the coding sequences; and (c) genetically engineered host cells which contain such vectors or have been engineered to contain and/or

express a nucleic acid sequence of the invention, *e.g.*, any of the foregoing coding sequences operatively associated with a regulatory element which directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art which drive and regulate expression. The invention further includes fragments of any of the DNA sequences disclosed herein.

The nucleic acid molecules may encode or act as antisense molecules, useful, for example, in protective sequence regulation, and/or as hybridization probes and/or as primers in amplification reactions of protective nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for protective sequence regulation. Still further, such molecules may be used as components of diagnostic methods whereby, for example, the presence of a particular allele involved in a condition, disorder, or disease involving cell death may be detected.

The protective nucleic acids of the invention can be readily obtained, for example, by standard sequencing and the sequences provided herein.

As will be appreciated by those skilled in the art, DNA sequence polymorphisms of a protective sequence will exist within a population of individual organisms (*e.g.*, within a human population). Such polymorphisms may exist, for example, among individuals within a population due to natural allelic variation. Such polymorphisms include ones that lead to changes in amino acid sequence. An allele is one of a group of alternative forms of a gene that occur at a given genetic locus.

As used herein, the phrase "allelic variant" refers to a nucleic acid that occurs at a given locus or to a gene product encoded by that nucleic acid. Such natural allelic variations can typically result in 1-5% variance in the nucleic acid of a given gene. Sequencing the gene of interest in a number of different individuals can identify alternative alleles. Using hybridization probes to identify the same genetic locus in a variety of individuals can readily carry this out.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising any of up to six open reading frames which may or may not encode a polypeptide of the invention. For example, the terms "gene" and "recombinant gene" refer to nucleic acid molecules encoding any of the open reading frames shown in Figures 4-13, and

described in Tables 2-11, respectively. The term can further include nucleic acid molecules comprising upstream and/or exon/intron sequences and structures.

## TABLE 2

## OPEN READING FRAMES FOR CNI-00718

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 39 Nucleotide | 202-240 of Seq. Id. No. 1 | 2 |
| | 12 Amino Acid | | 3 |
| 2 | 45 Nucleotide | 315-359 of Seq. Id. No. 1 | 4 |
| | 14 Amino Acid | | 5 |
| 3 | 51 Nucleotide | 356-406 of Seq. Id. No. 1 | 6 |
| | 16 Amino Acid | | 7 |
| 4 | 42 Nucleotide | 385-426 of Seq. Id. No. 1 | 8 |
| | 13 Amino Acid | | 9 |
| 5 | 15 Nucleotide | 423-437 of Seq. Id. No. 1 | 10 |
| | 4 Amino Acids | | 11 |
| 6 | 12 Nucleotide | 467-478 of Seq. Id. No.1 | 12 |
| | 3 Amino Acid | | 13 |
| 7 | 27 Nucleotide | 483-509 of Seq. Id. No. 1 | 14 |
| | 8 Amino Acid | | 15 |
| 8 | 51 Nucleotide | 597-647 of Seq. Id. No. 1 | 16 |
| | 16 Amino Acid | | 17 |
| 9 | 30 Nucleotide | 685-714 of Seq. Id. No. 1 | 18 |
| | 9 Amino Acids | | 19 |
| 10 | 221 Nucleotide | 704-925 of Seq. Id. No. 1 | 20 |
| | 73 Amino Acid | | 21 |
| 11 | 69 Nucleotide | 715-783 of Seq. Id. No. 1 | 22 |
| | 22 Amino Acid | | 23 |
| 12 | 57 Nucleotide | 727-783 of Seq. Id. No. 1 | 24 |
| | 18 Amino Acid | | 25 |
| 13 | 18 Nucleotide | 735-752 of Seq. Id. No. 1 | 26 |
| | 5 Amino Acids | | 27 |
| 14 | 30 Nucleotide | 891-920 of Seq. Id. No. 1 | 28 |
| | 9 Amino Acid | | 29 |
| 15 | 339 Nucleotide | 954-1292 of Seq. Id. No. 1 | 30 |
| | 112 Amino Acid | | 31 |
| 16 | 63 Nucleotide | 997-1059 of Seq. Id. No. 1 | 32 |
| | 20 Amino Acid | | 33 |
| 17 | 207 Nucleotide | 1086-1292 of Seq. Id. No. 1 | 34 |
| | 68 Amino Acids | | 35 |
| 18 | 72 Nucleotide | 1221-1292 of Seq. Id. No. 1 | 36 |
| | 23 Amino Acid | | 37 |
| 19 | 24 Nucleotide | 1335-1358 of Seq. Id. No. 1 | 38 |
| | 7 Amino Acid | | 39 |

| | | | |
|---|---|---|---|
| 20 | 21 Nucleotide | 1367-1387 of Seq. Id. No. 1 | 40 |
| | 6 Amino Acid | | 41 |
| 21 | 36 Nucleotide | 1439-1474 of Seq. Id. No. 1 | 42 |
| | 11 Amino Acids | | 43 |
| 22 | 183 Nucleotide | 1461-1643 of Seq. Id. No. 1 | 44 |
| | 60 Amino Acid | | 45 |
| 23 | 99 Nucleotide | 1541-1639 of Seq. Id. No. 1 | 46 |
| | 32 Amino Acid | | 47 |
| 24 | 18 Nucleotide | 1626-1643 of Seq. Id. No. 1 | 48 |
| | 5 Amino Acid | | 49 |
| 25 | 12 Nucleotide | 1632-1643 of Seq. Id. No. 1 | 50 |
| | 3 Amino Acids | | 51 |
| 26 | 21 Nucleotide | 1684-1704 of Seq. Id. No. 1 | 52 |
| | 6 Amino Acid | | 53 |
| 27 | 18 Nucleotide | 1725-1742 of Seq. Id. No. 1 | 54 |
| | 5 Amino Acids | | 55 |
| 28 | 27 Nucleotide | 1747-1773 of Seq. Id. No. 1 | 56 |
| | 8 Amino Acids | | 57 |

## TABLE 3

## OPEN READING FRAMES FOR CNI-00722

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 15 Nucleotide | 242-256 of Seq. Id. No. 58 | 59 |
| | 4 Amino Acid | | 60 |
| 2 | 27 Nucleotide | 301-327 of Seq. Id. No. 58 | 61 |
| | 8 Amino Acid | | 62 |
| 3 | 12 Nucleotide | 316-327 of Seq. Id. No. 58 | 63 |
| | 3 Amino Acid | | 64 |
| 4 | 51 Nucleotide | 385-435 of Seq. Id. No. 58 | 65 |
| | 16 Amino Acid | | 66 |
| 5 | 33 Nucleotide | 446-478 of Seq. Id. No. 58 | 67 |
| | 10 Amino Acid | | 68 |
| 6 | 15 Nucleotide | 478-492 of Seq. Id. No. 58 | 69 |
| | 4 Amino Acid | | 70 |
| 7 | 135 Nucleotide | 498-632 of Seq. Id. No. 58 | 71 |
| | 44 Amino Acid | | 72 |
| 8 | 57 Nucleotide | 576-632 of Seq. Id. No. 58 | 73 |
| | 18 Amino Acid | | 74 |
| 9 | 96 Nucleotide | 632-727 of Seq. Id. No. 58 | 75 |
| | 31 Amino Acid | | 76 |
| 10 | 93 Nucleotide | 635-727 of Seq. Id. No. 58 | 77 |
| | 30 Amino Acid | | 78 |
| 11 | 51 Nucleotide | 714-764 of Seq. Id. No. 58 | 79 |
| | 16 Amino Acids | | 80 |
| 12 | 57 Nucleotide | 754-810 of Seq. Id. No. 58 | 81 |
| | 19 Amino Acids | | 82 |

## TABLE 4

## OPEN READING FRAMES FOR CNI-00725

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 21 Nucleotide | 67-87 of Seq. Id. No. 83 | 84 |
| | 6 Amino Acid | | 85 |
| 2 | 39 Nucleotide | 187-225 of Seq. Id. No. 83 | 86 |
| | 12 Amino Acid | | 87 |
| 3 | 48 Nucleotide | 258-305 of Seq. Id. No. 83 | 88 |
| | 15 Amino Acid | | 89 |
| 4 | 75 Nucleotide | 262-336 of Seq. Id. No. 83 | 90 |
| | 24 Amino Acid | | 91 |
| 5 | 99 Nucleotide | 333-431 of Seq. Id. No. 83 | 92 |
| | 32 Amino Acids | | 93 |
| 6 | 12 Nucleotide | 359-370 of Seq. Id. No. 83 | 94 |
| | 3 Amino Acid | | 95 |
| 7 | 54 Nucleotide | 378-431 of Seq. Id. No. 83 | 96 |
| | 17 Amino Acid | | 97 |
| 8 | 45 Nucleotide | 482-526 of Seq. Id. No. 83 | 98 |
| | 14 Amino Acids | | 99 |
| 9 | 63 Nucleotide | 619-681 of Seq. Id. No. 83 | 100 |
| | 20 Amino Acid | | 101 |
| 10 | 42 Nucleotide | 640-681 of Seq. Id. No. 83 | 102 |
| | 13 Amino Acids | | 103 |
| 11 | 116 Nucleotide | 805-920 of Seq. Id. No. 83 | 104 |
| | 38 Amino Acids | | 105 |

## TABLE 5

## OPEN READING FRAMES FOR CNI-00726

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 444 Nucleotide | 23-466 of Seq. Id. No. 106 | 107 |
| | 147 Amino Acid | | 108 |
| 2 | 24 Nucleotide | 111-134 of Seq. Id. No. 106 | 109 |
| | 7 Amino Acid | | 110 |
| 3 | 15 Nucleotide | 138-152 of Seq. Id. No. 106 | 111 |
| | 4 Amino Acid | | 112 |
| 4 | 318 Nucleotide | 149-466 of Seq. Id. No. 106 | 113 |
| | 105 Amino Acid | | 114 |
| 5 | 42 Nucleotide | 163-204 of Seq. Id. No. 106 | 115 |
| | 13 Amino Acids | | 116 |
| 6 | 294 Nucleotide | 173-466 of Seq. Id. No. 106 | 117 |
| | 97 Amino Acid | | 118 |
| 7 | 30 Nucleotide | 201-230 of Seq. Id. No. 106 | 119 |
| | 9 Amino Acid | | 120 |
| 8 | 12 Nucleotide | 232-243 of Seq. Id. No. 106 | 121 |
| | 3 Amino Acid | | 122 |
| 9 | 177 Nucleotide | 290-466 of Seq. Id. No. 106 | 123 |
| | 58 Amino Acids | | 124 |
| 10 | 36 Nucleotide | 312-347 of Seq. Id. No. 106 | 125 |
| | 11 Amino Acids | | 126 |
| 11 | 18 Nucleotide | 352-369 of Seq. Id. No. 106 | 127 |
| | 5 Amino Acid | | 128 |
| 12 | 63 Nucleotide | 404-466 of Seq. Id. No. 106 | 129 |
| | 20 Amino Acid | | 130 |
| 13 | 60 Nucleotide | 407-466 of Seq. Id. No. 106 | 131 |
| | 19 Amino Acid | | 132 |
| 14 | 45 Nucleotide | 422-466 of Seq. Id. No. 106 | 133 |
| | 14 Amino Acids | | 134 |
| 15 | 27 Nucleotide | 624-650 of Seq. Id. No. 106 | 135 |
| | 8 Amino Acids | | 136 |
| 16 | 72 Nucleotide | 1006-1077 of Seq. Id. No. 106 | 137 |
| | 23 Amino Acid | | 138 |
| 17 | 57 Nucleotide | 1224-1280 of Seq. Id. No. 106 | 139 |
| | 18 Amino Acid | | 140 |
| 18 | 48 Nucleotide | 1335-1382 of Seq. Id. No. 106 | 141 |
| | 15 Amino Acid | | 142 |

| | | | |
|---|---|---|---|
| 19 | 15 Nucleotide | 1382-1396 of Seq. Id. No. 106 | 143 |
| | 4 Amino Acids | | 144 |
| 20 | 78 Nucleotide | 1492-1569 of Seq. Id. No. 106 | 145 |
| | 25 Amino Acid | | 146 |
| 21 | 33 Nucleotide | 1514-1546 of Seq. Id. No. 106 | 147 |
| | 10 Amino Acid | | 148 |
| 22 | 156 Nucleotide | 1670-1825 of Seq. Id. No. 106 | 149 |
| | 51 Amino Acid | | 150 |
| 23 | 30 Nucleotide | 1819-1848 of Seq. Id. No. 106 | 151 |
| | 9 Amino Acids | | 152 |
| 24 | 69 Nucleotide | 1827-1895 of Seq. Id. No. 106 | 153 |
| | 22 Amino Acids | | 154 |
| 25 | 63 Nucleotide | 1833-1895 of Seq. Id. No. 106 | 155 |
| | 20 Amino Acids | | 156 |
| 26 | 66 Nucleotide | 1951-2016 of Seq. Id. No. 106 | 157 |
| | 21 Amino Acids | | 158 |

## TABLE 6

## OPEN READING FRAMES FOR CNI-00727

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 45 Nucleotide | 237-281 of Seq. Id. No. 159 | 160 |
| | 14 Amino Acid | | 161 |
| 2 | 27 Nucleotide | 255-281 of Seq. Id. No. 159 | 162 |
| | 8 Amino Acid | | 163 |
| 3 | 12 Nucleotide | 395-406 of Seq. Id. No. 159 | 164 |
| | 3 Amino Acid | | 165 |
| 4 | 45 Nucleotide | 403-447 of Seq. Id. No. 159 | 166 |
| | 14 Amino Acid | | 167 |
| 5 | 48 Nucleotide | 419-466 of Seq. Id. No. 159 | 168 |
| | 15 Amino Acids | | 169 |
| 6 | 27 Nucleotide | 454-480 of Seq. Id. No. 159 | 170 |
| | 8 Amino Acid | | 171 |
| 7 | 39 Nucleotide | 610-648 of Seq. Id. No. 159 | 172 |
| | 12 Amino Acid | | 173 |
| 8 | 165 Nucleotide | 658-822 of Seq. Id. No. 159 | 174 |
| | 54 Amino Acid | | 175 |
| 9 | 132 Nucleotide | 691-822 of Seq. Id. No. 159 | 176 |
| | 43 Amino Acids | | 177 |
| 10 | 123 Nucleotide | 700-822 of Seq. Id. No. 159 | 178 |
| | 40 Amino Acid | | 179 |
| 11 | 111 Nucleotide | 712-822 of Seq. Id. No. 159 | 180 |
| | 36 Amino Acid | | 181 |
| 12 | 57 Nucleotide | 945-1001 of Seq. Id. No. 159 | 182 |
| | 18 Amino Acid | | 183 |
| 13 | 18 Nucleotide | 952-969 of Seq. Id. No. 159 | 184 |
| | 5 Amino Acids | | 185 |
| 14 | 15 Nucleotide | 962-976 of Seq. Id. No. 159 | 186 |
| | 4 Amino Acid | | 187 |
| 15 | 99 Nucleotide | 973-1071 of Seq. Id. No. 159 | 188 |
| | 32 Amino Acid | | 189 |
| 16 | 12 Nucleotide | 1071-1082 of Seq. Id. No. 159 | 190 |
| | 3 Amino Acid | | 191 |
| 17 | 63 Nucleotide | 1131-1193 of Seq. Id. No. 159 | 192 |
| | 20 Amino Acid | | 193 |
| 18 | 42 Nucleotide | 1152-1193 of Seq. Id. No. 159 | 194 |
| | 13 Amino Acids | | 195 |
| 19 | 12 Nucleotide | 1165-1176 of Seq. Id. No. 159 | 196 |
| | 3 Amino Acids | | 197 |

## TABLE 7

## OPEN READING FRAMES FOR CNI-00728

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 129 Nucleotide | 30-158 of Seq. Id. No. 198 | 199 |
| | 42 Amino Acid | | 200 |
| 2 | 69 Nucleotide | 70-138 of Seq. Id. No. 198 | 201 |
| | 22 Amino Acid | | 202 |
| 3 | 42 Nucleotide | 117-158 of Seq. Id. No. 198 | 203 |
| | 13 Amino Acid | | 204 |
| 4 | 39 Nucleotide | 187-225 of Seq. Id. No. 198 | 205 |
| | 12 Amino Acid | | 206 |
| 5 | 33 Nucleotide | 193-225 of Seq. Id. No. 198 | 207 |
| | 10 Amino Acid | | 208 |
| 6 | 24 Nucleotide | 202-225 of Seq. Id. No. 198 | 209 |
| | 7 Amino Acid | | 210 |
| 7 | 15 Nucleotide | 225-239 of Seq. Id. No. 198 | 211 |
| | 4 Amino Acid | | 212 |
| 8 | 21 Nucleotide | 331-351 of Seq. Id. No. 198 | 213 |
| | 6 Amino Acid | | 214 |
| 9 | 42 Nucleotide | 384-425 of Seq. Id. No. 198 | 215 |
| | 13 Amino Acid | | 216 |
| 10 | 60 Nucleotide | 404-463 of Seq. Id. No. 198 | 217 |
| | 19 Amino Acid | | 218 |
| 11 | 15 Nucleotide | 536-550 of Seq. Id. No. 198 | 219 |
| | 4 Amino Acid | | 220 |
| 12 | 39 Nucleotide | 626-664 of Seq. Id. No. 198 | 221 |
| | 12 Amino Acid | | 222 |
| 13 | 102 Nucleotide | 689-790 of Seq. Id. No. 198 | 223 |
| | 33 Amino Acid | | 224 |
| 14 | 60 Nucleotide | 731-790 of Seq. Id. No. 198 | 225 |
| | 19 Amino Acid | | 226 |
| 15 | 87 Nucleotide | 738-824 of Seq. Id. No. 198 | 227 |
| | 28 Amino Acid | | 228 |
| 16 | 180 Nucleotide | 910-1089 of Seq. Id. No. 198 | 229 |
| | 59 Amino Acid | | 230 |
| 17 | 99 Nucleotide | 991-1089 of Seq. Id. No. 198 | 231 |
| | 32 Amino Acid | | 232 |
| 18 | 27 Nucleotide | 1063-1089 of Seq. Id. No. 198 | 233 |
| | 8 Amino Acid | | 234 |

| | | | |
|---|---|---|---|
| 19 | 150 Nucleotide | 1124-1273 of Seq. Id. No. 198 | 235 |
| | 49 Amino Acid | | 236 |
| 20 | 54 Nucleotide | 1143-1196 of Seq. Id. No. 198 | 237 |
| | 17 Amino Acid | | 238 |
| 21 | 87 Nucleotide | 1187-1273 of Seq. Id. No. 198 | 239 |
| | 28 Amino Acid | | 240 |
| 22 | 42 Nucleotide | 1242-1283 of Seq. Id. No. 198 | 241 |
| | 13 Amino Acid | | 242 |
| 23 | 15 Nucleotide | 1306-1320 of Seq. Id. No. 198 | 243 |
| | 4 Amino Acids | | 244 |
| 24 | 139 Nucleotide | 1382-1466 of Seq. Id. No. 198 | 245 |
| | 46 Amino Acids | | 246 |

## TABLE 8

## OPEN READING FRAMES FOR CNI-00729

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 1386 Nucleotide | 140-1525 of Seq. Id. No. 247 | 248 |
| | 461 Amino Acid | | 249 |
| 2 | 75 Nucleotide | 213-287 of Seq. Id. No. 247 | 250 |
| | 24 Amino Acid | | 251 |
| 3 | 69 Nucleotide | 219-287 of Seq. Id. No. 247 | 252 |
| | 22 Amino Acid | | 253 |
| 4 | 39 Nucleotide | 357-395 of Seq. Id. No. 247 | 254 |
| | 12 Amino Acid | | 255 |
| 5 | 72 Nucleotide | 417-488 of Seq. Id. No. 247 | 256 |
| | 23 Amino Acid | | 257 |
| 6 | 1068 Nucleotide | 458-1525 of Seq. Id. No. 247 | 258 |
| | 355 Amino Acid | | 259 |
| 7 | 12 Nucleotide | 477-488 of Seq. Id. No. 247 | 260 |
| | 3 Amino Acid | | 261 |
| 8 | 1038 Nucleotide | 488-1525 of Seq. Id. No. 247 | 262 |
| | 345 Amino Acid | | 263 |
| 9 | 918 Nucleotide | 608-1525 of Seq. Id. No. 247 | 264 |
| | 305 Amino Acid | | 265 |
| 10 | 888 Nucleotide | 638-1525 of Seq. Id. No. 247 | 266 |
| | 295 Amino Acid | | 267 |
| 11 | 75 Nucleotide | 699-773 of Seq. Id. No. 247 | 268 |
| | 24 Amino Acid | | 269 |
| 12 | 663 Nucleotide | 863-1525 of Seq. Id. No. 247 | 270 |
| | 220 Amino Acid | | 271 |
| 13 | 462 Nucleotide | 1064-1525 of Seq. Id. No. 247 | 272 |
| | 153 Amino Acid | | 273 |
| 14 | 432 Nucleotide | 1094-1525 of Seq. Id. No. 247 | 274 |
| | 143 Amino Acid | | 275 |
| 15 | 423 Nucleotide | 1103-1525 of Seq. Id. No. 247 | 276 |
| | 140 Amino Acid | | 277 |
| 16 | 339 Nucleotide | 1187-1525 of Seq. Id. No. 247 | 278 |
| | 112 Amino Acid | | 279 |
| 17 | 63 Nucleotide | 1290-1352 of Seq. Id. No. 247 | 280 |
| | 20 Amino Acid | | 281 |
| 18 | 33 Nucleotide | 1320-1352 of Seq. Id. No. 247 | 282 |
| | 10 Amino Acid | | 283 |
| 19 | 238 Nucleotide | 1422-1659 of Seq. Id. No. 247 | 284 |
| | 79 Amino Acid | | 285 |

| | | | |
|---|---|---|---|
| 20 | 78 Nucleotide | 1448-1525 of Seq. Id. No. 247 | 286 |
| | 25 Amino Acid | | 287 |
| 21 | 67 Nucleotide | 1593-1659 of Seq. Id. No. 247 | 288 |
| | 22 Amino Acids | | 289 |
| 22 | 41 Nucleotide | 1619-1659 of Seq. Id. No. 247 | 290 |
| | 13 Amino Acids | | 291 |

## TABLE 9

## OPEN READING FRAMES FOR CNI-00730

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 429 Nucleotide | 128-556 of Seq. Id. No. 292 | 293 |
| | 142 Amino Acid | | 294 |
| 2 | 30 Nucleotide | 264-293 of Seq. Id. No. 292 | 295 |
| | 9 Amino Acid | | 296 |
| 3 | 18 Nucleotide | 276-293 of Seq. Id. No. 292 | 297 |
| | 5 Amino Acid | | 298 |
| 4 | 21 Nucleotide | 435-455 of Seq. Id. No. 292 | 299 |
| | 6 Amino Acid | | 300 |
| 5 | 51 Nucleotide | 474-524 of Seq. Id. No. 292 | 301 |
| | 16 Amino Acids | | 302 |
| 6 | 51 Nucleotide | 506-556 of Seq. Id. No. 292 | 303 |
| | 16 Amino Acid | | 304 |
| 7 | 33 Nucleotide | 524-556 of Seq. Id. No. 292 | 305 |
| | 10 Amino Acid | | 306 |
| 8 | 51 Nucleotide | 573-623 of Seq. Id. No. 292 | 307 |
| | 16 Amino Acid | | 308 |
| 9 | 74 Nucleotide | 649-722 of Seq. Id. No. 292 | 309 |
| | 24 Amino Acids | | 310 |

TABLE 10

OPEN READING FRAMES FOR CNI-00731

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 48 Nucleotide | 56-103 of Seq. Id. No. 311 | 312 |
| | 15 Amino Acid | | 313 |
| 2 | 24 Nucleotide | 80-103 of Seq. Id. No. 311 | 314 |
| | 7 Amino Acid | | 315 |
| 3 | 18 Nucleotide | 86-103 of Seq. Id. No. 311 | 316 |
| | 5 Amino Acid | | 317 |
| 4 | 99 Nucleotide | 107-205 of Seq. Id. No. 311 | 318 |
| | 32 Amino Acid | | 319 |
| 5 | 72 Nucleotide | 199-270 of Seq. Id. No. 311 | 320 |
| | 23 Amino Acids | | 321 |
| 6 | 36 Nucleotide | 235-270 of Seq. Id. No. 311 | 322 |
| | 11 Amino Acid | | 323 |
| 7 | 98 Nucleotide | 267-364 of Seq. Id. No. 311 | 324 |
| | 32 Amino Acids | | 325 |

**TABLE 11**

**OPEN READING FRAMES FOR CNI-00732**

| OPEN READING FRAME NUMBER | LENGTH | LOCATION | SEQUENCE ID. NO. |
|---|---|---|---|
| 1 | 24 Nucleotide | 23-46 of Seq. Id. No. 326 | 327 |
| | 7 Amino Acid | | 328 |
| 2 | 63 Nucleotide | 100-162 of Seq. Id. No. 326 | 329 |
| | 20 Amino Acid | | 330 |
| 3 | 108 Nucleotide | 418-525 of Seq. Id. No. 326 | 331 |
| | 35 Amino Acid | | 332 |
| 4 | 18 Nucleotide | 611-628 of Seq. Id. No. 326 | 333 |
| | 5 Amino Acid | | 334 |
| 5 | 51 Nucleotide | 671-721 of Seq. Id. No. 326 | 335 |
| | 16 Amino Acids | | 336 |
| 6 | 36 Nucleotide | 686-721 of Seq. Id. No. 326 | 337 |
| | 11 Amino Acid | | 338 |
| 7 | 30 Nucleotide | 727-756 of Seq. Id. No. 326 | 339 |
| | 9 Amino Acid | | 340 |
| 8 | 152 Nucleotide | 895-1046 of Seq. Id. No. 326 | 341 |
| | 50 Amino Acids | | 342 |

Alternative or differential splicing of a gene that encodes any of the open reading frames shown in Figures 4-13 can also generate an alternative or differential protective sequence product. For example, a gene that generates one of the protective sequence products shown in Figures 4-13 may be encoded by 4 out of 6 exons that comprise the entire gene; alternative or differential splicing of the gene can generate other protective sequence products that are encoded by 1, 2, 3, 4, 5, or 6 of the exons in the gene (Lewin, 2000, Genes VII, Oxford University Press, 702-705). The present invention also includes nucleic acid molecules comprising nucleic acids that separately encode these alternative or differential protective sequence products.

In a specific embodiment, the nucleic acid molecules comprise nucleic acids that encode an open reading frame of at least 3 contiguous amino acid residues from a full-length protein. In alternate embodiments, the nucleic acid molecules comprise an open reading frame which encodes at least about 5, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acid residues of a protein.

The sequence obtained from clones containing partial coding sequences or non-coding sequences can be used to obtain the entire coding region by using the RACE method, for example (Chenchik, et al., 1995, CLONTECHniques (X) 1: 5-8; Barnes, 1994, Proc. Natl. Acad. Sci. USA 91: 2216-2220; and Cheng et al., Proc. Natl. Acad. Sci. USA 91: 5695-5699). Oligonucleotides can be designed based on the sequence obtained from the partial clone that can amplify a reverse transcribed mRNA encoding the entire coding sequence. Alternatively, probes can be used to screen cDNA libraries prepared from an appropriate cell or cell line in which the protective sequence is transcribed.

With respect to allelic variants of protective sequences associated with a condition, disorder, or disease involving cell death, any and all such nucleotide variations and resulting amino acid polymorphisms or variations which are the result of natural allelic variation of the protective sequence are intended to be within the scope of the present invention. Such allelic variants include, but are not limited to, ones that do not alter the functional activity of the protective sequence product.

With respect to the cloning of additional allelic variants of the isolated protective sequence and homologues and orthologs from other species (*e.g.*, guinea pig, cow, mouse), the isolated protective sequences disclosed herein may be labeled and used to screen

a cDNA library constructed from mRNA obtained from appropriate cells or tissues (*e.g.*, brain) derived from the organism (*e.g.*, guinea pig, cow and mouse) of interest. The hybridization conditions used generally should be of a lower stringency when the cDNA library is derived from an organism different from the type of organism from which the labeled sequence was derived, and can routinely be determined based on, *e.g.*, relative relatedness of the target and reference organisms.

Alternatively, the labeled fragment may be used to screen a genomic library derived from the organism of interest, again, using appropriately stringent conditions. Appropriate stringency conditions are well known to those of skill in the art as discussed above, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions, *see*, for example, Sambrook, *et al.*, 1989, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y.; and Ausubel, *et al.*, 1989-1999, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y., both of which are incorporated herein by reference in their entirety.

Additionally, the cloning of homologs and orthologs of the isolated protective sequence from other species (e.g. mouse) could also occur using the knowledge of syntenic regions and/or genes. Syntenic genes are genes which are believed to be located on the same chromosome because they are lost along with a marker gene which is known to be located on that chromosome. There are well-established genetic maps of specific chromosome regions that show syntenic regions between chromosomes of humans and other species that can be utilized, by one skilled in the art, for this purpose.

Further, a protective sequence allelic variant may be isolated from, for example, human nucleic acid, by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of amino acid sequences within the protective sequence product of interest. The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from, for example, human or non-human cell lines or tissue known or suspected to express a wild type or mutant protective sequence allele. In one embodiment, the allelic variant is isolated from an individual who has a condition, disorder, or disease involving cell death. Such variants are described in the examples below.

The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences of a protective nucleic acid sequence. The PCR fragment may then be used to isolate a full-length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to isolate genomic clones via the screening of a genomic library.

PCR technology also may be utilized to isolate full-length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction. The hybrid may be digested with RNAase H and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see *e.g.*, Sambrook *et al.*, 1989, *supra*, or Ausubel *et al.*, *supra*.

In cases where the isolated protective sequence is the normal, or wild type gene, this gene may be used to isolate mutant alleles of the protective sequence. Such an isolation is preferable in processes and disorders that are known or suspected to have a genetic basis. Mutant alleles may be isolated from individuals either known or suspected to have a genotype which contributes to symptoms of conditions, disorders, or diseases involving cell death. Mutant alleles and mutant allele products may then be utilized in the therapeutic and diagnostic assay systems described below.

A cDNA of the mutant protective sequence may be isolated, for example, by using PCR, a technique well known to those of skill in the art. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying the mutant allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal protective sequence. Using these two primers, the product is then amplified via PCR, cloned into a suitable vector and subjected to DNA sequence analysis

through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant protective sequence to that of the normal protective sequence, the mutation(s) responsible for the loss or alteration of function of the mutant gene product can be ascertained.

Alternatively, a genomic or cDNA library can be constructed and screened using DNA or RNA, respectively, from a tissue known to or suspected of expressing the protective sequence of interest in an individual suspected of or known to carry the mutant allele. The normal protective sequence or any suitable fragment thereof may then be labeled and used as a probed to identify the corresponding mutant allele in the library. The clone containing this protective sequence may then be purified through methods routinely practiced in the art, and subjected to sequence analysis as described above in this Section.

Additionally, an expression library can be constructed utilizing DNA isolated from or cDNA synthesized from a tissue known to or suspected of expressing the protective sequence of interest in an individual suspected of or known to carry the mutant allele. In this manner, protective sequence products made by the tissue containing the putative mutant alleles may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal protective sequence product, as described, below, in Section 5.3 (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed protective sequence product with altered function (*e.g.*, as a result of a missense mutation), a polyclonal set of antibodies are likely to cross-react with the mutant protective sequence product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis as described in this Section, above.

The invention also includes nucleic acid molecules, preferably DNA molecules that are the complements of the nucleic acids of the preceding paragraphs.

In certain embodiments, the protective nucleic acid molecules of the invention are present as part of protective nucleic acid molecules comprising nucleic acid sequences which do not contain heterologous (*e.g.*, cloning vector or expression vector) sequences. In other embodiments, the protective nucleic acid molecules of the invention further comprise vector sequences, *e.g.*, cloning vectors or expression vectors.

## 5.2 Protein Products of the Protective Sequences

Protective sequence products or fragments thereof of the invention can be prepared for a variety of uses, including but not limited to, prophylactic or therapeutic modulators of protective sequence product function, for the generation of antibodies, diagnostic assays, or for the identification of other cellular or extracellular protective sequence products involved in the regulation of conditions, disorders, or diseases involving cell death.

The protective sequence products of the invention include, but are not limited to, human protective sequence products and non-human protective sequence products, *e.g.*, mammalian (such as bovine or guinea pig), protective sequence products.

Protective sequence products of the invention, sometimes referred to herein as a "protective sequence protein" or "protective sequence polypeptide," includes those gene products encoded by any of up to six translational reading frames of the protective sequence sequences depicted in Table 1, as well as gene products encoded by other human allelic variants and non-human variants of protective sequence products which can be identified by the methods herein described. Among such protective sequence product variants are protective sequence products comprising amino acid residues encoded by polymorphisms of such protective sequence products.

In addition, protective sequence products of the invention may include proteins that represent functionally equivalent gene products. Functionally equivalent protective sequence products may include, for example, protective sequence products encoded by one of the nucleic acid molecules described in Section 5.1, above. In preferred embodiments, such functionally equivalent protective sequence products are naturally occurring gene products. Functionally equivalent protective sequence products also include gene products which retain at least one of the biological activities of the protective sequence products described above, and/or which are recognized by and bind to antibodies (polyclonal or monoclonal) directed against protective sequence products of the invention.

Equivalent protective sequence products may contain deletions, including internal deletions, additions, including additions yielding fusion proteins, or substitutions of amino acid residues within and/or adjacent to the amino acid sequence encoded by the protective sequence sequences described, above, in Section 5.1. Generally, deletions will be

deletions of single amino acid residues, or deletions of no more than about 2, 3, 4, 5, 10 or 20 amino acid residues, either contiguous or non-contiguous. Generally, additions or substitutions, other than additions which yield fusion proteins, will be additions or substitutions of single amino acid residues, or additions or substitutions of no more than about 2, 3, 4, 5, 10 or 20 amino acid residues, either contiguous or non-contiguous. Preferably, these modifications result in a "silent" change, in that the change produces a protective sequence product with the same activity as the original protective sequence product. However, nucleic acid changes resulting in amino acid additions or substitutions may also be made for the purpose of modifying the protective sequence product in order to generally enhance their use as therapeutic agents or components for assays, such modifications to include, but not be limited to, stabilizing the product against degradation, enhancing pharmacokinetic properties, modifying site tropisms at the level of cells, tissues, organs, or organisms.

Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine; positively charged (basic) amino acids include arginine, lysine and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Additionally, non-natural amino acids, including, but not limited to, D-amino acids may be used.

Alternatively, where alteration of function is desired, addition(s), deletion(s) or non-conservative alterations can produce altered, including reduced-activity, protective sequence products. Such alterations can, for example, alter one or more of the biological functions of the protective sequence product. Further, such alterations can be selected so as to generate protective sequence products which include, but are not limited to, products which are better suited for expression, scale up, *etc.* in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

Protective sequence products of the invention also include gene products generated by alternative or differential splicing patterns of a gene that encodes for the

peptides shown in Figures 4-13. An isolated gene often includes alternating exons and introns; as a result, the same gene can generate a variety of gene products by alternative or differential forms of splicing.

Protein fragments and/or peptides of the invention may comprise at least as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an antibody directed to the protein). Examples of such protein fragments and/or peptides of the invention are shown by the open reading frames of the protective sequences shown in Figures 4-13, and described in Tables 2-11, respectively. In one nonlimiting embodiment of the invention, such protein fragments or peptides comprise at least about 3 contiguous amino acid residues from a full-length protein. In alternate embodiments, the protein fragments and peptides of the invention can comprise about 5, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acid residues of a protein.

Peptides and/or proteins corresponding to one or more domains of the protein as well as fusion proteins in which a protein, or a portion of a protein such as a truncated protein or peptide or a protein domain, is fused to an unrelated protein are also within the scope of this invention. Such proteins and peptides can be designed on the basis of the nucleic acids disclosed in Section 5.1, above. Fusion proteins include, but are not limited to, IgFc fusions which stabilize the protein or peptide and prolong half-life *in vivo*; or fusions to any amino acid sequence which allows the fusion protein to be anchored to the cell membrane; or fusions to an enzyme, fluorescent protein, luminescent protein or a epitope tagged protein or peptide which provides a marker function.

The protein sequences described above can include a domain, which comprises a protein transduction domain which targets the protective sequence product for delivery to various tissues and more particularly across the brain blood barrier, using, for example, the protein transduction domain of human immunodeficiency virus TAT protein (Schwarze *et al.*, 1999, Science 285: 1569-72).

The protein sequences described above can include a domain, which comprises a signal sequence that targets the gene product for secretion. As used herein, a signal sequence includes a peptide of at least about 15 or 20 amino acid residues in length which occurs at the N-terminus of secretory and membrane-bound proteins and which

contains at least about 70% hydrophobic amino acid residues such as alanine, leucine, isoleucine, phenylalanine, proline, tyrosine, tryptophan or valine. In a preferred embodiment, a signal sequence contains at least about 10 to 40 amino acid residues, preferably about 19-34 amino acid residues and has at least about 60-80%, more preferably 65-75% and more preferably at least about 70% hydrophobic residues. A signal sequence serves to direct a protein containing such a sequence to a lipid bilayer.

A signal sequence of a polypeptide of the invention can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids, which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence (that is, "immature" polypeptides), as well as to the signal sequences themselves and to the polypeptides in the absence of a signal sequence (*i.e.*, the "mature" cleavage products). It is to be understood that polypeptides of the invention can further comprise polypeptides comprising any signal sequence having characteristics as described above and a mature polypeptide sequence.

In one embodiment, a nucleic acid sequence encoding a signal sequence of the invention can be operably linked in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

Finally, the proteins of the invention also include protein sequences wherein domains encoded by any transcriptional or post-transcriptional, and/or translational or post-translational modifications, or fragments thereof, have been deleted. The polypeptides of the invention can further comprise posttranslational modifications, including, but not limited to glycosylations, acetylations and myrisalations.

The protective sequence products, peptide fragments thereof and fusion proteins thereof may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protective sequence products, polypeptides, peptides, fusion peptide and fusion polypeptides of the invention by expressing nucleic acid containing protective sequence sequences are described herein. Methods that are well known to those skilled in the art can be used to construct expression vectors containing protective sequence product coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* genetic recombination. *See*, for example, the techniques described in Sambrook, *et al.*, 1989, *supra*, and Ausubel, *et al.*, 1989, *supra*. Alternatively, RNA capable of encoding protective sequence product sequences may be chemically synthesized using, for example, synthesizers. *See*, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, ed., IRL Press, Oxford.

A variety of host-expression vector systems may be utilized to express the protective sequence product coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the protective sequence product of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing protective sequence product coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the protective sequence product coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the protective sequence product coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing protective sequence product coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionine promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the protective sequence product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of protective sequence product or for raising antibodies to protective sequence product, for example, vectors which direct the expression of high levels of fusion protein products which are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO J. 2:1791), in which the protective sequence product coding sequence may be ligated individually into the vector in frame with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke and Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned protective sequence product can be released from the GST moiety.

In an insect system, *Autographa californica*, nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The protective sequence product coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of protective sequence product coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (*e.g.*, *see* Smith, *et al.*, 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the protective sequence product coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader

sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing protective sequence products in infected hosts. (*See*, *e.g.*, Logan and Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted protective sequence product coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire protective sequence, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the protective sequence coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (*see* Bittner, *et al.*, 1987, *Methods in Enzymol.* 153:516-544).

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3 and WI38. Additional host cells derived from neuronal tissue include, but are not limited to, PC-12 cells and primary dissociated neurons which are removed from the brain and grown in culture.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the protective sequence product may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the protective sequence product. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the protective sequence product.

A number of selection systems may be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48:2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, *Cell* 22:817) genes can be employed in $tk^-$, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:3567; O'Hare, *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, *J. Mol. Biol.* 150:1); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, *Gene* 30:147).

Alternatively, the expression characteristics of an endogenous protective sequence within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous protective sequence. For example, an endogenous protective sequence which is normally "transcriptionally silent", *i.e.*, a protective sequence which is normally not expressed, or is

expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed protective sequence product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous protective sequence may be activated by insertion of a promiscuous regulatory element which works across cell types.

Methods, which are well known to those skilled in the art, can be used to construct vectors containing the protective sequence operatively associated with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, and synthetic techniques. *See*, for example, the techniques described in Sambrook, *et al.*, 1992, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Greene Publishing Associates & Wiley Interscience, N.Y.

The protective sequences may be associated operatively with a variety of different promoter/enhancer elements. The expression elements of these vectors may vary in their strength and specificities. Depending on the host/vector system utilized, any one of a number of suitable transcription and translation elements may be used. The promoter may be in the form of the promoter that is associated naturally with the gene of interest. Alternatively, the DNA may be positioned under the control of a recombinant or heterologous promoter, *i.e.*, a promoter that is not associated normally with that gene. For example, tissue specific promoter/enhancer elements may be used to regulate the expression of the transferred DNA in specific cell types. Examples of transcriptional control regions which exhibit tissue specificity which have been described and could be used, include, but are not limited to: choline acetyltransferase (ChAT) gene control region which is active in cholinergic cells in the brain (Lonnerberg *et al.*, 1996, JBC 271:33358-65; Lonnerberg *et al.*, 1995, PNAS 92: 4046-50; Ibenez and Perrson, 1991 Eur. J. Neurosci. 3: 1309-15), mouse Thy-1.2 gene control region which is active in adult neurons including hippocampus, thalamus, cerebellum, cortex, RGC, DRG, and MN in the brain (Caroni, 1997, J Neurosci. Meth. 71: 3-9; Vidal *et al.*, 1990, EMBO J 9: 833-40), neuron specific enolase (NSE) gene control region which is active in pan-neuronal, neuron specific, deep layers of cerebral and neocortex (not in white matter) areas of the brain (Hannas-Djebbara *et al.*, 1997, Brain Res. Mol. Brain Res. 46: 91-9; Peel *et al.*, 1997, Gene Therapy 4: 16-24; Twyman *et al.*, 1997, J Mol Neurosci 8: 63-73;

Forss-Petter *et al.,* 1990, Neuron 5:187-97), elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:42S-51S); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122); immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adams *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444); albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276); alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58); alpha-1-antitrypsin gene control region which is active in liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171); beta-globin gene control region which is active in myeloid cells (Magram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378). Promoters isolated from the genome of viruses which grow in mammalian cells (*e.g.*, CMV, RSV, vaccinia virus 7.5K, SV40, HSV, adenoviruses MLP, and MMTV LTR promoters) may be used, as well as promoters produced by recombinant DNA or synthetic techniques. Further, promoters specifically activated within bone, *i.e.*, the osteocalcin promoter, which is specifically activated within cells of osteoblastic lineage, may be used to target expression of nucleic acids within bone cells.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous protective sequence, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

Alternatively, utilizing an antibody specific for the fusion protein being expressed may readily purify any fusion protein. For example, a system described by Janknecht, *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8972-8976). In

this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto $Ni^{2+}$-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The protective sequence products can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys and chimpanzees may be used to generate transgenic animals. The term "transgenic," as used herein, refers to animals expressing protective sequences from a different species (*e.g.*, mice expressing human protective sequences), as well as animals which have been genetically engineered to overexpress endogenous (*i.e.*, same species) sequences or animals which have been genetically engineered to no longer express endogenous protective sequences (*i.e.*, "knock-out" animals), and their progeny.

Any technique known in the art may be used to introduce a protective sequence transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Hoppe and Wagner, 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten, *et al.*, 1985, *Proc. Natl. Acad. Sci., USA* 82:6148-6152); gene targeting in embryonic stem cells (Thompson, *et al.*, 1989, *Cell* 56:313-321); electroporation of embryos (Lo, 1983, *Mol. Cell. Biol.* 3:1803-1814); and sperm-mediated gene transfer (Lavitrano *et al.*, 1989, *Cell* 57:717-723) (For a review of such techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115, 171-229).

Any technique known in the art may be used to produce transgenic animal clones containing a protective sequence transgene, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal or adult cells induced to quiescence (Campbell, *et al.*, 1996, *Nature* 380:64-66; Wilmut, *et al.*, *Nature* 385:810-813).

The present invention provides for transgenic animals which carry a protective sequence transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene also

may be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.* (Lasko, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:6232-6236). The regulatory sequences required for such a cell-type specific activation will depend on the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the cerebral transgene be integrated into the chromosomal site of the endogenous protective sequence, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleic acids homologous to the endogenous protective sequence are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleic acid of the endogenous protective sequence. The transgene also may be selectively introduced into a particular cell type, thus inactivating the endogenous protective sequence in only that cell type, by following, for example, the teaching of Gu, *et al.* (Gu, *et al.*, 1994, *Science* 265, 103-106). The regulatory sequences required for such a cell-type specific inactivation will depend on the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant protective sequence may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis and RT-PCR (reverse transcriptase PCR). Samples of protective sequence-expressing tissue also may be evaluated immunocytochemically using antibodies specific for the transgene product.

Protective proteins can be used, *e.g.*, to treat cell death-related conditions, disorders, or diseases. Such protective sequence products include, but are not limited to, soluble derivatives such as peptides or polypeptides corresponding to one or more domains of the protective sequence product which are modified such that they are deleted for one or more hydrophobic domains. Alternatively, antibodies to the protein or anti-idiotypic antibodies which mimic the protective sequence product (including Fab fragments), modulators, antagonists or agonists can be used to treat cell death-related conditions, disorders, or

diseases involving the protective sequence product. In yet another approach, nucleotide constructs encoding such protective sequence products can be used to genetically engineer host cells to express such protective sequence products *in vivo*; these genetically engineered cells can function as "bioreactors" in the body delivering a continuous supply of protective sequence product, peptides and soluble polypeptides.

### 5.3 Antibodies to the Protective Sequence Products

Described herein are methods for the production of antibodies capable of specifically recognizing one or more protective sequence product epitopes or epitopes of conserved variants or peptide fragments of the protective sequence products of the invention. Further, antibodies that specifically recognize mutant forms of the protective sequence products of the invention are encompassed by the invention. The terms "specifically bind" and "specifically recognize" refer to antibodies which bind to protective sequence product epitopes involved in conditions, disorders, or diseases involving cell death at a higher affinity than they bind to protective sequence product epitopes not involved in such conditions, disorders, or diseases (*e.g.*, random epitopes).

Such antibodies may include, but are not limited to, polyclonal antibodies, monoclonal antibodies (mAbs), human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of a protective sequence product in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal levels of protective sequence products, and/or for the presence of abnormal forms of such protective sequence products. Such antibodies also may be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.4.2, for the evaluation of the effect of test compounds on protective sequence product levels and/or activity. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described below, in Section 5.4.1.3., to evaluate, for example, the normal and/or engineered cells prior to their introduction into the patient.

Antibodies derived from the protective sequence or protective sequence product, including, but not limited to, antibodies and anti-idiotypic antibodies that mimic activity or function additionally may be used in methods for inhibiting abnormal protective sequence product activity. Thus, such antibodies may, therefore, be utilized as part of treatment methods for protective sequence product-mediated conditions, disorders, or diseases.

For the production of antibodies against a protective sequence, various host animals may be immunized with a protective sequence or protective sequence product, or a portion thereof. Such host animals may include, but are not limited to, rabbits, mice and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including, but not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as protective sequence product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized with protective sequence product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, *Nature* 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor *et al.*, 1983, *Immunology Today* 4:72; Cole *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, *e.g.*, Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison (1985) *Science* 229:1202-1207; Oi et al. (1986) *Bio/Techniques* 4:214; U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced, for example, using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently

undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) *Bio/technology* 12:899-903).

In addition, techniques developed for the production of "chimeric antibodies" (Morrison, *et al.*, 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger, *et al.*, 1984, *Nature* 312:604-608; Takeda, *et al.*, 1985, *Nature*, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, *e.g.*, Cabilly *et al.*, U.S. Patent No. 4,816,567; and Boss *et al.*, U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.)

In addition, techniques have been developed for the production of humanized antibodies. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarily determining regions (CDRs). The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest", Kabat, E. *et al.*, U.S. Department of Health and Human Services (1983) ). Briefly, humanized antibodies are antibody molecules from non-human species having one or more

CDRs from the non-human species and a framework region from a human immunoglobulin molecule.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423-426; Huston, *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward, *et al.*, 1989, *Nature* 334:544-546) can be adapted to produce single chain antibodies against protective sequence products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, such fragments include, but are not limited to: the $F(ab')_2$ fragments, which can be produced by pepsin digestion of the antibody molecule and the Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed (Huse, *et al.*, 1989, *Science* 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

### 5.4 Uses of the Protective Sequences, Protective Sequence Products and Antibodies

Described herein are various uses and applications of protective sequences, protective sequence products, including peptide fragments and fusion proteins thereof and of antibodies and anti-idiotypic antibodies derived from the protective sequence products and peptide fragments thereof. The application relates to compositions and methods for the treatment of conditions, disorders, or diseases involving cell death. Such applications include, but are not limited to, the prophylactic or therapeutic use of protective sequences which, when introduced into a cell predisposed to undergo cell death or in the process of dying, to prevent, delay, or rescue a cell, cells, tissue, organs, or organisms from dying, as described below in Section 5.4.1

Additionally, such applications include methods for the treatment of conditions, disorders, or diseases involving cell death, including, but not limited to, those associated with the central nervous system including neurological and psychiatric conditions, disorders, or diseases, and others as described below, in Section 5.4.1.1, and for the

identification of compounds which modulate the expression of the protective sequence and/or the synthesis or activity of the protective sequence product, as described below, in Section 5.4.1. Such compounds can include, for example, other cellular products that are involved in such processes as the regulation of cell death. These compounds can be used, for example, in the amelioration of conditions, disorders, or diseases involving cell death.

One example of the type of injury that can cause cell death in neuronal cells is stroke, which often is the result of ischemic injury. A relatively broad time window (8 hours to perhaps several days or longer) exists between the onset of ischemic injury (*i.e.* cessation or marked reduction in blood flow) before most neural cells actually die. There are many complex pathways and perhaps hundreds of different signaling molecules which are likely to be involved, leaving many different intervention points each with the potential to prevent, delay, arrest and reverse the cell death program. These delayed biochemical intervention points represent ideal clinical intervention points as they correspond to the time period during which most stroke patients present for medical treatment.

Many current medications for the treatment of stroke affect the physical and biochemical events that are acutely related to the initial onset of stroke, and, thus, must be administered soon after the biochemical cascades begin. These approaches all suffer from the necessity of administering the drugs within a very brief time window following a stroke. However, many stroke patients do not even realize that they have suffered from a stroke until a time point at which many of the current treatments are ineffective. This is because many stroke patients often do not present at the emergency room prior to the passing of at least 13 hours from the onset of the stroke. The methods and compounds of the present invention, however, can be administered during the broader time window between stroke and the onset of the pathways leading to cell death.

In addition to stroke, a variety of other conditions, disorders, and diseases lead to the activation of the same biochemical cascades which lead to neuronal cell death in stroke. There is growing evidence that numerous other disease states that induce cell death programs are related to those induced by stroke. Cell death programs have been increasingly implicated in Alzheimer's disease, a well-known neurodegenerative condition which leads to substantial loss of specific neuronal populations in the neocortex and hippocampus. Vascular dementia (multi-infarct dementia) is another disorder in which stroke-like cell death pathways

are active. In vascular dementia, a repetitive process of small blood vessel diseases induces regional brain cell death, leading to a progressive loss of cognitive abilities. A partial list of other brain diseases which activate brain cell death pathways similar to those observed in stroke include, but are not limited to, Parkinson's disease, traumatic injury, Down's syndrome, Huntington's disease, HIV infection and intracranial infections.

One notable example from the preceding list is physical trauma to the nervous system. Although such trauma can be caused by a multitude of different physical insults to the head, neck, spine and other parts of the nervous system, all result in focal damage to, and death of, neural tissue and its component cells. Focally damaged areas behave similarly to stroke-induced infarcts in that a wider area of neural damage and death, a penumbra, is induced via biochemical and cellular mechanisms which are similar or identical to those occurring in stroke.

While, for clarity, the uses described in this section are primarily uses related to conditions, disorders, or diseases involving cell death, it is to be noted that each of the diagnostic and therapeutic treatments described herein can be additionally utilized in connection with other defects associated with the protective sequences of the invention.

Additionally, described herein are various applications of protective sequences, protective sequence products, genes, gene products, and/or their regulatory elements, including, but not limited to, prognostic and diagnostic evaluation of conditions, disorders, or diseases as described below in Section 5.4.1.1.

A variety of methods can be employed for the diagnostic and prognostic evaluation of conditions, disorders, or diseases involving cell death and for the identification of subjects having a predisposition to such conditions, disorders, or diseases.

Since protective sequences or protective sequence products need not normally be involved in all conditions, disorders, or diseases involving cell death, methods of the invention include, for example, modulating the expression of the protective sequence and/or the activity of the protective sequence product for the treatment of conditions, disorders, or diseases involving cell death which are normally mediated by some other gene.

For cell death related conditions, disorders, or diseases in which the protective sequences or protective sequence products are involved normally, such diagnostic and prognostic methods may, for example, utilize reagents such as the protective nucleic acids

described in Section 5.1, and antibodies directed against protective sequence products, including peptide fragments thereof, as described, above, in Section 5.3.

Specifically, such reagents may be used, for example, for:

(1) the detection of the presence of protective sequence mutations, or the detection of either over- or under-expression of the protective sequence relative to wild-type levels of expression;

(2) the detection of over- or under-abundance of protective sequence products relative to wild-type abundance of the protective sequence product; and

(3) the detection of an aberrant level of protective sequence product activity relative to wild-type protective sequence product activity levels.

Protective nucleic acids can, for example, be used to diagnose a condition, disorder, or disease involving cell death using, for example, the techniques for mutation/polymorphism detection described above in Section 5.1.

Mutations at a number of different genetic loci may lead to phenotypes related to conditions, disorders, or diseases involving cell death. Ideally, the treatment of patients suffering from such conditions, disorders, or diseases will be designed to target the particular genetic loci containing the mutation mediating the condition, disorder, or disease. Genetic polymorphisms have been linked to differences in drug effectiveness. Thus, identification of alterations in protective sequence, protein or gene flanking regions can be utilized in pharmacogenetic methods to optimize therapeutic drug treatments.

In one embodiment of the present invention, therefore, alterations, *i.e.*, polymorphisms, in the protective sequence or protein encoded by genes comprising such polymorphisms, are associated with a drug or drugs' efficacy, tolerance or toxicity, and may be used in pharmacogenomic methods to optimize therapeutic drug treatments, including therapeutic drug treatments for one of the conditions, disorders, or diseases described herein contained in Section 5.4.1.1, *e.g.*, central nervous system conditions, disorders, or diseases. Such polymorphisms can be used, for example, to refine the design of drugs by decreasing the incidence of adverse events in drug tolerance studies, *e.g.*, by identifying patient subpopulations of individuals who respond or do not respond to a particular drug therapy in efficacy studies, wherein the subpopulations have a polymorphism associated with drug responsiveness or unresponsiveness. The pharmacogenomic methods of the present invention

also can provide tools to identify new drug targets for designing drugs and to optimize the use of already existing drugs, *e.g.*, to increase the response rate to a drug and/or to identify and exclude non-responders from certain drug treatments (*e.g.*, individuals having a particular polymorphism associated with unresponsiveness or inferior responsiveness to the drug treatment) or to decrease the undesirable side effects of certain drug treatments and/or to identify and exclude individuals with marked susceptibility to such side effects (*e.g.*, individuals having a particular polymorphism associated with an undesirable side effect to the drug treatment).

In an embodiment of the present invention, polymorphisms in the protective sequence or flanking this sequence, or variations in protective sequence expression, or activity, *e.g.*, variations due to altered methylation, differential splicing or post-translational modification of the protective sequence product, may be utilized to identify an individual having a disease or condition resulting from a disorder involving cell death and thus define the most effective and safest drug treatment. Assays such as those described herein may be used to identify such polymorphisms or variations in protective sequence expression or activity. Once a polymorphism in the protective sequence or in a flanking sequence in linkage disequilibrium with a disorder-causing allele, or a variation in protective sequence expression has been identified in an individual, an appropriate drug treatment can be prescribed to the individual.

For the detection of protective sequence mutations or polymorphisms, any nucleated cell can be used as a starting source for genomic nucleic acid. For the detection of protective sequence expression or protective sequence products, any cell type or tissue in which the protective sequence is expressed may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.4.1.4. Peptide detection techniques are described, below, in Section 5.4.1.5.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits. The invention therefore also encompasses kits for detecting the presence of a polypeptide or nucleic acid of the invention in a biological sample (*i.e.*, a test sample). Such kits can be used, *e.g.*, to determine if a subject is suffering from or is at increased risk of developing a condition, disorder, or disease associated with a disorder-causing allele, or aberrant expression or activity of a polypeptide of the invention. For

example, the kit can comprise a labeled compound or agent capable of detecting the polypeptide or mRNA or DNA or protective sequence sequences, *e.g.*, encoding the polypeptide in a biological sample. The kit can comprise further a means for determining the amount of the polypeptide or mRNA in the sample (*e.g.*, an antibody that binds the polypeptide or an oligonucleotide probe that binds to DNA or mRNA encoding the polypeptide). Kits can also include instructions for observing that the tested subject is suffering from, or is at risk of developing, a condition, disorder, or disease associated with aberrant expression of the polypeptide if the amount of the polypeptide or mRNA encoding the polypeptide is above or below a normal level, or if the DNA correlates with presence of an allele which causes a condition, disorder, or disease.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (*e.g.*, attached to a solid support) which binds to a polypeptide of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or to the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide (*e.g.*, a detectably labeled oligonucleotide) which hybridizes to a nucleic acid sequence encoding a polypeptide of the invention, or (2) a pair of primers useful for amplifying a nucleic acid molecule encoding a polypeptide of the invention.

The kit also can comprise, for example, one or more buffering agents, preservatives or protein stabilizing agents. The kit also can comprise components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate). The kit can contain also a control sample or a series of control samples that can be assayed and compared to the test sample. Each component of the kit usually is enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a condition, disorder, or disease associated with polymorphisms which correlate with alleles which cause conditions, disorders, or diseases involving cell death, and/or aberrant levels of mRNA, polypeptides or activity.

Additionally, the application relates to the compositions and methods for the development of screening assays for the identification of compounds, described in Section

5.4.2 below, which interact with or modulate protective sequences, protective sequence products, genes, gene products, and/or their regulatory elements.

### 5.4.1 Composition and Methods for the Treatment of Conditions, Disorders, or Diseases Involving Cell Death

This application relates to compositions and methods for the treatment of conditions, disorders, or diseases involving cell death. Such applications include, but are not limited to, the prophylactic or therapeutic use of protective sequences, protective sequence products, genes, gene products, or the regulatory elements, target sequences, or variants of any of the aforementioned sequences or products, which, when introduced into a cell predisposed to undergo cell death or in the process of dying, prevent, delay, or rescue a cell, cells, tissue, organs, or organisms from dying. The application further relates to the methods and compositions whereby a condition, disorder, or disease involving cell death, including but not limited to, the conditions, disorders, or diseases mentioned in Section 5.4.1.1, may be treated wherein such methods can comprise administering antibodies, antisense molecules or sequences, ribozyme molecules, or other inhibitors or modulators directed against such protective sequences, protective sequence products, genes, gene products, or the regulatory elements, target sequences, or variants of any of the aforementioned sequences or products.

The application relates to compositions and methods for those instances whereby the condition, disorder, or disease involving cell death results from protective sequence mutations, such methods can comprise supplying the subject with a nucleic acid molecule encoding an unimpaired protective sequence product such that an unimpaired protective sequence product is expressed and the cell, cells, tissue, organ, organism displaying symptoms of the condition, disorder, or disease is prevented, delayed, or rescued from death.

In another embodiment of methods for the treatment of conditions, disorders, or diseases involving cell death resulting from protective sequence mutations, such methods can comprise supplying the subject with a cell comprising a nucleic acid molecule which encodes an unimpaired protective sequence product such that the cell expresses the unimpaired protective sequence product and the cell, cells, tissue, organ, or organism displaying symptoms of the condition, disorder, or disease is prevented, delayed, or rescued

from death.

In cases in which a loss of normal protective sequence product function results in the development of a condition, disorder, or disease involving cell death, an increase in protective sequence product activity would facilitate progress towards an asymptomatic state in individuals exhibiting a deficient level of protective sequence expression and/or gene product activity. Methods for enhancing the expression or synthesis of protective sequence product can include, for example, methods such as those described below, in Section 5.4.1.3.

Alternatively, symptoms of a condition, disorder, or disease involving cell death may be prevented, delayed, or rescued by administering a compound which decreases the level of protective sequence expression and/or gene product activity. Methods for inhibiting or reducing the level of protective sequence product synthesis or expression can include, for example, methods such as those described in Section 5.4.1.2.

In cases where the development of a condition, disorder, or disease involving cell death is due to a sequence or gene other than a protective sequence, modulating, including but not limited to, mimicking, agonizing, or antagonizing the expression of a protective sequence and/or the activity of a protective sequence product, or their regulatory elements, can be used for the treatment of the condition, disorder, or disease involving cell death. This is because protective sequences are nucleic acid molecules comprising nucleic acid sequences which, when introduced into a cell predisposed to undergo cell death, prevent, delay, or rescue such cell death relative to a corresponding cell into which no exogenous protective sequence has been introduced.

The proteins and peptides which may be used in the methods of the invention include synthetic (*e.g.*, recombinant or chemically synthesized) proteins and peptides, as well as naturally occurring proteins and peptides. The proteins and peptides may have both naturally occurring and non-naturally occurring amino acid residues (*e.g.*, D-amino acid residues) and/or one or more non-peptide bonds (*e.g.*, imino, ester, hydrazide, semicarbazide, and azo bonds). The proteins or peptides may also contain additional chemical groups (*i.e.*, functional groups) present at the amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptide is enhanced. Exemplary functional groups include hydrophobic groups (*e.g.* carbobenzoxyl, dansyl, and t-butyloxycarbonyl, groups), an acetyl group, a 9-fluorenylmethoxy-carbonyl group and

macromolecular carrier groups (*e.g.*, lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates) including peptide groups. Additional proteins and peptides which may be used in the methods of the invention include those described in WO 99/59615, which is herein incorporated by reference in its entirety.

#### 5.4.1.1 Examples of Conditions, Disorders, or Diseases Involving Cell Death

The types of conditions, disorders, or diseases which can be prevented, delayed, or rescued by the compounds and methods of the present invention include, but are not limited to, those associated with the central nervous system including neurological and psychiatric conditions, disorders, or diseases; those of the peripheral nervous system; conditions, disorders, or diseases caused by physical injury; conditions, disorders, or diseases of the blood vessels or heart; conditions, disorders, or diseases of the respiratory system; neoplastic conditions, disorders, or diseases; conditions, disorders, or diseases of blood cells; conditions, disorders, or diseases of the gastrointestinal tract; conditions, disorders, or diseases of the liver; conditions, disorders, or diseases of the pancreas; conditions, disorders, or diseases of the kidney; conditions, disorders, or diseases of the ureters, urethra or bladder; conditions, disorders, or diseases of the male genital system; conditions, disorders, or diseases of the female genital tract; conditions, disorders, or diseases of the breast; conditions, disorders, or diseases of the endocrine system; conditions, disorders, or diseases of the thymus or pineal gland; conditions, disorders, or diseases of the skin or mucosa; conditions, disorders, or diseases of the musculoskeletal system; conditions, disorders, or diseases causing a fluid or hemodynamic derangement; inherited conditions, disorders, or diseases; conditions, disorders, or diseases of the immune system or spleen; conditions, disorders, or diseases caused by a nutritional disease; and conditions, disorders, or diseases typically occurring in infancy or childhood.

Conditions, disorders, or diseases involving the central nervous system include, but are not limited to, common pathophysiologic complications such as increased intracraneal pressure and cerebral herniation, septic embolism, cerebral edema, suppurative endovasculitis and hydrocephalus; infections such as meningitis, acute meningitis, acute lymphocytic meningitis, chronic meningitis, purulent meningitis, syphilitic gumma,

encephalitis, cerebral abscess, epidural abscess, subdural abscess, brain abscess, viral encephalitis, acute viral encephalitis, encephalomeningitis, aseptic meningitis, post-infectious encephalitis, subacute encephalitis, chronic encephalitis, chronic meningitis, chronic encephalomeningitis, slow virus diseases and unconventional agent encephalopathies; protozoal infections such as malaria, toxoplasmosis, amebiasis and trypanosomiasis; rickettsial infections such as typhus and Rocky Mountain spotted fever; metazoal infections such as echinococcosis and cysticercosis; vascular diseases such as ischemic encephalopathy, cerebral infarction, intracranial hemorrhage, intraparenchymal hemorrhage, subarachnoid hemorrhage, mixed intraparenchymal and subarachnoid hemorrhage; conditions involving the eye such as macular degeneration, glaucoma, retinopathy of prematurity, retinitis pigmentosa, diabetic retinopathy, or other traumatic injuries to the retina or optic nerve; trauma such as epidural hematoma, subdural hematoma, parenchymal injuries; tumors such as primary intrachranial tumors, astrocytoma, oligodendroglioma, ependymoma, medulloblastoma and meningioma; degenerative diseases such as Altzheimer's disease, Huntington's disease, Parkinsonism, idiopathic Parkinson's disease and motor neuron disease; demyelinating diseases such as multiple sclerosis; nutritional, environmental and metabolic conditions, disorders, or diseases.

Conditions, disorders, or diseases of the peripheral nervous system include, but are not limited to, peripheral neuropathy, acute idiopathic polyneuropathy, diabetic neuropathy and peripheral nerve tumors.

Conditions, disorders, or diseases caused by physical injury include, but are not limited to, the direct, indirect, immediate, or delayed effects of: changes in temperature such as frostbite and thermal burns; an increase in atmospheric pressure such as air blast or immersion blast caused by an explosion; a decrease in atmospheric pressure such as caisson disease or high-altitude hypoxia; mechanical violence from penetrating or non-penetrating traumatic injury; electromechanical energy such as radiation injury from either charged particles or electromagnetic waves; electrocution or non-ionizing radiation such as radio waves, microwaves, laser light or ultrasound.

Conditions, disorders, or diseases of the blood vessels or heart include, but are not limited to, hypertension (high blood pressure), heart failure; ischemic or atherosclerotic heart disease; myocardial infarction; cardiac arrest; hypertensive heart disease; cor

pulmonale; valvular heart disease such as that caused by rheumatic fever, aortic valve stenosis, mitral annulus calcification, carcinoid heart disease, nonbacterial thrombotic endocarditis, or nonbacterial verrucous endocarditis; infectious endocarditis caused by organisms including, but not limited to, Streptococcus species, Staphylococcus species, enterococci, pneumococci, gram-negative rods, Candida species, Aspergillus species, or culture-negative endocarditis; congenital heart disease such as atrial septal defect, ventricular septal defect, patent ductus arteriosis, coarctation of the aorta, Tetralogy of Fallot, tricuspid atresia, pulmonary stenosis or atresia, aortic stenosis or atresia, bicuspid aortic valve, or hypoplastic left heart syndrome; cardiomyopathy; pericarditis; pericardial effusion; rheumatoid heart disease; congenital anomalies of the blood vessels; arteriosclerosis including, but not limited to atherosclerosis, Monckeberg's medial calcific stenosis, hyaline arteriosclerosis, or hyperplastic arteriosclerosis; one or more of the vasculidities including, but not limited to, polyarteritis nodosa, hypersensitivity angiitis, Wegener's granulomatosis, giant cell (temporal) arteritis, Takayasu's arteritis, Kawasaki's disease, thromboangiitis obliterans, infectious vasculitis, Raynaud's disease; arteriosclerotic aortic aneurysm; syphilitic aortic aneurysm; dissecting aortic aneurysm; varicose veins; thrombophlebitis; lymphangitis; lymphedema; telangiectases; or arteriovenous malformations (AVM).

Conditions, disorders, or diseases of the respiratory system include, but are not limited to, pulmonary congestion; heart failure; embolism; infarction; pulmonary hypertension; adult respiratory distress syndrome (ARDS); obstructive lung disease; restrictive lung disease; chronic obstructive pulmonary disease; asthma; sarcoidosis; diffuse interstitial or infiltrative lung diseases including, but not limited to, idiopathic pulmonary fibrosis, pneumoconiosis, hypersensitivity pneumonitis, Goodpasture's syndrome, idiopathic pulmonary hemosiderosis, collagen-vascular diseases, or pulmonary eosinophilia; serofibrinous pleuritis; suppurative pleuritis; hemorrhagic pleuritis; pleural effusions; pneumothorax; hemothorax or pneumohemothorax.

Neoplastic conditions, disorders, or diseases include, but are not limited to, benign tumors composed of one parenchymal cell type such as fibromas, myxomas, lipomas, hemangiomas, meningiomas, leiomyomas, adenomas, nevi, moles, or papillomas; benign mixed tumors derived from one germ layer such as a mixed tumor of salivary gland origin; benign mixed tumors derived from more than one germ layer such as a teratoma; primary

malignant tumors or metastases of malignant tumors composed of one parenchymal cell type such as sarcomas, Ewing's tumor, leukemia, myeloma, histiocytosis X, Hodgkin's disease, lymphomas, carcinomas, melanomas, bronchial adenoma, small cell lung cancer, or seminoma; primary malignant tumors or metastases of mixed malignant tumors derived from one germ layer such as Wilms' tumor or malignant mixed salivary gland tumor; primary malignant tumor or metastases of mixed malignant tumors derived from one germ layer such as malignant teratoma or teratocarcinoma; undifferentiated benign tumor or undifferentiated malignant tumor.

Conditions, disorders, or diseases of blood cells include, but are not limited to, anemia due to one or more of the following conditions: acute blood loss, chronic blood loss, hemolytic anemia, sickle cell disease, thalassemia syndromes, autoimmune hemolytic anemia, traumatic anemia, or diminished erythropoesis from megaloblastic anemia, iron deficiency, aplastic anemia, idiopathic bone marrow failure; polycythemia; hemorrhagic diatheses related to increased vascular fragility; hemorrhagic diatheses related to a reduction in platelets; idiopathic or thrombotic thrombocytopenic purpura; hemorrhagic diatheses related to defective platelet function; hemorrhagic diatheses related to abnormalities in clotting factor(s); disseminated intravascular coagulation (DIC); neutropenia; agranulocytosis; leukocytosis; plasma cell dyscrasias such as myeloma, Waldenstrom's macroglobulinemia, or heavy-chain disease; or histiocytosis.

Conditions, disorders, or diseases of the gastrointestinal tract include, but are not limited to, congenital anomalies such as atresia, fistulas, or stenosis; periodontal disease; periapical disease; xerostomia; necrotizing sialometaplasia; esophageal rings or webs; hernia; Mallory-Weiss syndrome; esophagitis; diverticulosis; diverticulitis; scleroderma; esophageal varices; acute or chronic gastritis; peptic ulcer; gastric erosion or ulceration; ischemic bowel disease; infarction; embolism; Crohn's disease; obstruction from foreign bodies, hernia, adhesion, intussusception, or volvulus; ileus; megacolon; angoidysplasia; ulcerative colitis; psuedomembranous colitis; or polyps.

Conditions, disorders, or diseases of the liver include, but are not limited to, acute hepatic failure due to one of more of metabolic, circulatory, toxic, microbial, or neoplastic causes; chronic hepatic failure due to one or more of metabolic, circulatory, toxic, microbial, or neoplastic causes; hereditary hyperbilirubinemias; infarct; embolism; hepatic

circulation thrombosis or obstruction; fulminant hepatic necrosis; portal hypertension; alcoholic liver disease; post-necrotic cirrhosis; biliary cirrhosis; cirrhosis associated with alpha-1-antitrypsin deficiency; Wilson's disease; or Reye's syndrome.

Conditions, disorders, or diseases of the pancreas include, but are not limited to, congenital aberrant pancreas, congenital anomalies of pancreatic ducts, stromal fatty infiltration, pancreatic atrophy, acute hemorrhagic pancreatitis, chronic pancreatitis, chronic calcifying pancreatitis, chronic obstructive pancreatitis, pancreatic psuedocyst, diabetes mellitus, or gestational diabetes.

Conditions, disorders, or diseases of the kidney include, but are not limited to, congenital anomalies; polycystic renal disease; dialysis-associated cystic disease; glomerular disease, including, but not limited to, acute glomerulonephritis, acute proliferative glomerulonephritis, rapidly progressive glomerulonephritis, postinfectious rapidly progressive glomerulonephritis, Goodpasture's syndrome, idiopathic rapidly progressive glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis, lipoid nephrosis, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, focal proliferative glomerulonephritis, chronic glomerulonephritis, or hereditary nephritis; acute tubular necrosis; acute renal failure; tubulointerstitial diseases including, but not limited to, pyelonephritis, drug-induced interstitial nephritis, analgesic nephritis, urate nephropathy, hypercalcemia and nephrocalcinosis, hypokalemic nephropathy, myeloma-induced tubulointerstitial disease, radiation nephritis, immunologically medicated tubulointerstitial disease; hypertension; malignant hypertension; renal artery stenosis; renal diseases secondary to microangiopathic hemolytic anemia; atheroembolic renal disease; sickle cell disease nephropathy; diffuse cortical necrosis; renal infarcts; obstructive uropathy; or urolithiasis.

Conditions, disorders, or diseases of the ureters, urethra or bladder include, but are not limited to, congenital anomalies; inflammatory diseases; physical obstruction by causes including, but not limited to calculi, strictures, neoplasia, blood clot, or pregnancy; sclerosing retroperitonitis; acute cystitis; chronic cystitis; interstitial cystitis; emphysematous cystitis; eosinophilic cystitis; encrusted cystitis; fistula; or neurogenic bladder.

Conditions, disorders, or diseases of the male genital system include, but are not limited to, congenital anomalies; balanoposthitis; condyloma; phimosis; paraphimosis; dysplastic epithelial lesions; nonspecific epididymitis or orchitis; granulomatous orchitis;

torsion of the testis or its vascular supply; granulomatous prostatitis; acute or chronic prostatitis; or benign prostatic hyperplasia.

Conditions, disorders, or diseases of the female genital tract include, but are not limited to, congenital anomalies, lichen scleroses, acute cervicitis, chronic cervicitis, cervical polyps; acute endometritis; chronic endometritis; endometriosis; dysfunctional uterine bleeding; endometrial hyperplasia; senile cystic endometrial atrophy; salpingitis; polycystic ovary disease; pre-eclampsia or eclampsia (toxemia of pregnancy); placentitis; threatened abortion; or ectopic pregnancy.

Conditions, disorders, or diseases of the breast include, but are not limited to, congenital anomalies, acute mastitis, chronic mastitis, galactocele, granulomas, traumatic fat necrosis, mammary duct ectasia, fibrocystic disease, sclerosing adenitis, epithelial hyperplasia, hypertrophy, or gynecomastia.

Conditions, disorders, or diseases of the endocrine system include, but are not limited to, congenital anomalies; Sheehan's pituitary necrosis; empty sella syndrome; hyperthyroidism (thyrotoxicosis) from causes including, but not limited to, Graves' disease, toxic multinodular goiter, toxic adenoma, acute or subacute thyroiditis, TSH-secreting tumor, neonatal thyrotoxicosis, iatrogenic thyrotoxicosis; Hashimoto's thyroiditis; hypothyroidism (cretinism or myxedema) from causes including, but not limited to, surgical or radioactive ablation, primary idiopathic myxedema, iodine deficiency, goitrogenic agents, hypopituitarism, hypothalamic lesions, TSH resistance, subacute thyroiditis, or chronic thyroiditis; diffuse nontoxic simple or multinodular goiter; multiple endocrine neoplasia syndromes; primary or secondary hyperparathyroidism; chief cell hyperplasia; clear cell hyperplasia; hypoparathyroidism; pseudo- and pseudopseudohypoparathyrodism; Addison's disease; Waterhouse-Friderichsen syndrome; secondary adrenocortical insufficiency; Cushing's syndrome; Conn's syndrome; or congenital adrenal hyperplasia.

Conditions, disorders, or diseases of the skin or mucosa include, but are not limited to, melanocytic proliferative disorders; inflammatory dermatoses including, but not limited to, eczematous dermatitis, urticaria, erythema multiforme, cutaneous necrotizing vasculitis, cutaneous lupus erythematosus, graft-versus-host disease, panniculitis, acne vulgaris, rosacea, lichen planus, lichen sclerosus et atrophicus, pityriasis, psoriasis, or parapsoriasis; blistering diseases including, but not limited to, pemphigus, bullous

pemphigoid, dermatitis herpetiformis, or porphyria.

Conditions, disorders, or diseases of the musculoskeletal system include, but are not limited to, muscular atrophy; segmental necrosis; myositis; muscular dystrophy, including, but not limited to, Duchenne type, Becker type, Fascioscapulohumeral, Limb-Girdle, myotonic dystrophy, or ocular myopathy; congenital myopathies; myasthenia gravis; traumatic myositis ossificans; nodular fasciitis; desmoid tumors; palmar fibromatosis; congenital bone disorders including, but not limited to, osteogenesis imperfecta, achondroplasia, osteopetrosis, osteochondromatosis, endochondromatosis; osteomyelitis; fractures; osteoporosis; osteomalacia; bony changes secondary to hyperparathyroidism; Paget's disease; hypertrophic osteoarthropathy; fibrous dysplasia; or nonossifying fibroma.

Conditions, disorders, or diseases causing a fluid or hemodynamic derangement include, but are not limited to, systemic edema; anasarca; edema from increased hydrostatic pressure including, but not limited to congestive heart failure, cirrhosis of the liver, constrictive pericarditis, venous obstruction; edema from reduced oncotic pressure including, but not limited to, cirrhosis of the liver, malnutrition, protein-losing renal disease, protein-losing gastroenteropathy, protein loss through increased vascular permeability; edema from lymphatic obstruction including, but not limited to, cancer, inflammatory injury, surgical injury, traumatic injury, or radiation injury; edema from increased osmotic tension in the interstitial fluid including, but not limited to, sodium retention from excessive salt intake or increased renal sodium retention, reduced renal perfusion, acute or chronic renal failure, acute or chronic renal insufficiency; edema from increased endothelial permeability including, but not limited to, inflammation, shock, burns, trauma, allergic reaction, immunologic reaction, or adult respiratory distress syndrome; ascites; pericardial effusion; hydrothorax; hyperemia; hemorrhage; mural thrombus or occlusive thrombus diminishing or obstructing vascular flow; phlebothrombosis; blood clot; embolism; thromboembolism; disseminated intravascular coagulation (DIC); amniotic fluid infusion; amniotic fluid embolism; systemic embolism disease; septic embolism; fat embolism; pulmonary embolism; air gas embolism (caisson disease or decompression sickness); anemic (white) infarction; hemorrhagic (red) infarction; cerebral infarction; septic infarction; ischemia; cardiogenic shock from conditions including, but not limited to, myocardial infarction, cardiac arrest, cardiac rupture, cardiac tamponade, pulmonary embolism, cardiac valvular obstruction, or

cardiac arrhythmias; hypovolemic shock from conditions including, but not limited to, hemorrhage, vomiting, diarrhea, diaphoresis, extensive injury to bone or soft tissues, burns, or accumulation of intraperitoneal fluid; shock due to peripheral blood pooling from conditions including, but not limited to, spinal cord injury, general anesthesia, regional anesthesia, local anesthesia, drug-induced ganglionic or adrenergic blockade, gram-negative septicemia, or gram-positive septicemia; anaphylaxis, or disseminated intravascular coagulation (DIC).

Inherited conditions, disorders, or diseases include, but are not limited to, Down's syndrome, Edwards' syndrome, Patau's syndrome, other trisomies, Cri du Chat syndrome, Klinefelter's syndrome, XYY syndrome, Turner's syndrome, Multi-X female syndrome, hermaphrodism or pseudohermaphrodism, Marfan's syndrome, neurofibromatosis, vonHippel-Lindau disease, familial hypercholesterolemia, albinism, alkaptonuria, Fabry's disease, Fragile-X syndrome, Ehlers-Danlos syndromes, inherited neoplastic syndromes, inherited autosomal dominant conditions, Huntington's disease, Alport's disease, sickle-cell disease, thalessemia, tuberous sclerosis, vonWillebrand's disease, polycystic kidney disease, Pompe's disease, GM1-gangliosidosis; Tay-Sachs disease, Sandhoff-Jatzkewitz disease, metachromatic leukodystrophy, multiple sufatase deficiency, Krabbe's disease, Gaucher's disease, Niemann-Pick disease, all types of mucopolysaccharidoses, I-cell disease, Hurler's polydystrophy, fucosidosis, mannosidosis, aspartylglycosaminuria, Wolman's disease, or acid phosphatase deficiency, inherited autosomal recessive conditions, inherited sex-linked conditions.

Conditions, disorders, or diseases of the immune system or spleen include, but are not limited to, Type I hypersensitivity conditions (anaphylaxis and other basophil or mast cell mediated conditions), Type II hypersensitivity conditions (cytotoxic conditions involving phagocytosis or lysis of target cell), Type III hypersensitivity conditions (immune complex conditions involving antigen-antibody complexes), Type IV hypersensitivity conditions (cell-mediated conditions), transplant rejection, systemic lupus erythematosus, Sjogren's syndrome, CREST, scleroderma, polymyositis-dermatomyositis, mixed connective tissue disease, polyarteritis nodosa, amyloidosis, X-linked agammaglobulinemia, common variable immunodeficiency, isolated IgA deficiency, DiGeorge's syndrome, severe combined immunodeficiency, Wiscott-Aldrich syndrome, infection with HIV virus, acquired immune deficiency syndrome (AIDS), congenital anomalies of the immune system, hypersplenism,

splenomegaly, congenital anomalies of the spleen, congestive splenomegaly, infarcts, or splenic rupture.

Conditions, disorders, or diseases caused by a nutritional disease include, but are not limited to, marasmus, kwashiorkor, fat-soluble vitamin deficiency or toxicity (Vitamins A, D, E, or K), water-soluble vitamin deficiency or toxicity (thiamine, riboflavin, niacin, pyridoxine, folate, cobalamin, Vitamin C), mineral deficiency or toxicity (iron, calcium, magnesium, sodium, potassium, chloride, zinc, copper, iodine, cobalt, chromium, selenium, nickel, vanadium, manganese, molybdenum, rickets, osteomalacia, beriberi, hypoprothrombinemia, pellagra, megaloblastic anemia, scurvy, pernicious anemia, lack of gastric intrinsic factor, removal or pathophysiological functioning in the terminal ileum, microcytic anemia, or obesity.

Conditions, disorders, or diseases typically occurring in infancy or childhood include, but are not limited to, preterm birth, congenital malformations from genetic causes, congenital malformations from infectious causes, congenital malformations from toxic or teratogenic causes, congenital malformations from radiation, congenital malformations from idiopathic causes, small for gestational age infants, perinatal trauma, perinatal asphyxia, perinatal ischemia or hypoxia, birth injury, intracranial hemorrhage, deformations, respiratory distress syndrome of the newborn, atelectasis, hemolytic disease of the newborn, kernicterus, hydrops fetalis, congenital anemia of the newborn, icterus gravis, phenylketonuria, galactosemia, cystic fibrosis, hamartoma, or choristoma.

In another embodiment, the compounds and methods of the invention can be used to treat infections that cause cell death. The infections may be caused by bacteria; viruses; members of the family rickettsiae or chlamydia; fungi, yeast, hyphae or pseudohyphae; prions; protozoas; or metazoas.

Examples of aerobic or anaerobic bacteria which may cause such infections include, but are not limited to, gram-positive cocci, gram-positive bacilli (gram-positive rods), gram-negative cocci, gram-negative bacilli (gram-negative rods), Mycoplasma species, Ureaplasma species, Treponema species, Leptospira species, Borrelia species, Vibrio species, Mycobacteria species, members of Actinomycetes or L-forms (cell-wall deficient forms).

Examples of DNA, RNA or both DNA and RNA viruses which may cause such infections include, but are not limited to, members of the families adenoviridae,

parvoviridae, papovaviridae, herpesviridae, poxviridae, picornaviridae, orthomyxoviridae, paramyxoviridae, rhabdoviridae, bunyaviridae, arenaviridae, coronaviridae, retroviridae, reoviridae, togaviridae and caliciviridae.

Examples of members of the families rickettsiae or chlamydiae which may cause such infections include, but are not limited to, Rickettsia species, Rochalimaea species, Coxiella species or Chlamydia species.

Examples of fungi, yeast, hyphae or pseudohyphae which may cause such infections include, but are not limited to, members of Ascomycota, Basidiomycota, Zygomycota, or Deutoeromycota (Fungi Imperfecti); Candida species, Cryptococcus species, Torulopsis species, Rhodotorula species, Sporothrix species, Phialophora species, Cladosporium species, Xylohypha species, Blastomyces species, Histoplasma species, Coccidioides species, Paracoccidioides species, Geotrichum species, Aspergillus species, Rhizopus species, Mucor species, Pseudoallescheria species or Absidia species.

Examples of prions which may cause such infections include, but are not limited to, the causative agent of Creutzfeldt-Jakob Disease, the causative agent of Gerstmann-Straussler-Scheinker Disease, the causative agent of fatal familial insomnia, the causative agent of kuru, and the causative agent of bovine spongiform encephalopathy.

Examples of protozoa at any point in their life cycle which may cause such infections include, but are not limited to, Entamoeba species, Naegleria species, Acanthamoeba species, Pneumocystis species, Balantidium species, members of order Leptomyxida, Plasmodium species, Toxoplasma species, Leishmania species and Trypanosoma species.

Examples of metazoa at any point in their life cycle which may cause such infections include, but are not limited to, members of Platyhelminthes such as the organisms in Cestoda (tapeworms) or Trematoda (flukes); or members of Aschelminthes such as the organisms in Acanthocephala, Chaetognatha, Cycliophora, Gastrotricha, Nematoda or Rotifera.

In a further embodiment, the compounds and methods of the invention can be used to treat infections or disorders which cause cell death in organ systems including, but not limited to, blood vessels, heart, red blood cells, white blood cells, lymph nodes, spleen, respiratory system, oral cavity, gastrointestinal tract, liver and biliary tract, pancreas, kidney,

lower urinary tract, upper urinary tract and bladder, male sexual organs and genitalia, female sexual organs and genitalia, breast, thyroid gland, adrenal gland, parathyroid gland, skin, musculoskeletal system, bone marrow or bones.

In a further embodiment, the compounds and methods of the invention can be used to treat further physiological impacts on organs caused by the infections which induce cell death including, but not limited to, fever equal to or greater than 101.5 degrees Fahrenheit, a decrease or increase in pulse rate by more than 20 beats per minute, a decrease or increase in supine systolic blood pressure by more than 30 millimeters of mercury, an increase or decrease in respiratory rate by more than 8 breaths per minute, an increase or decrease in blood pH by more than 0.10 pH units, an increase or decrease in one or more serum electrolytes outside of the clinical laboratory's usual reference range, an increase or decrease in the partial pressure of arterial oxygen or carbon dioxide outside of the clinical laboratory's usual reference range, an increase or decrease in white or red blood cells outside of the laboratory's usual reference range, an acute confusional state such as delirium where delirium is defined by the American Psychiatric Association's DSM-IV Manual or a diminished level of consciousness or attention.

#### 5.4.1.2 Modulatory Antisense, Ribozyme and Triple Helix Approaches

In another embodiment, the types of conditions, disorders, or diseases involving cell death which may be prevented, delayed, or rescued by modulating protective sequence expression, protective sequence product activity, or their regulatory elements by using protective sequences in conjunction with well-known antisense, gene "knock-out," ribozyme and/or triple helix methods, are described. Among the compounds which may exhibit the ability to modulate the activity, expression or synthesis of the protective sequence, the protective sequence product, or its regulatory elements, including the ability to prevent, delay, or rescue a cell, cells, tissue, organ, or organism from the symptoms of a condition, disorder, or disease involving cell death are antisense, ribozyme and triple helix molecules. Such molecules may be designed to modulate, reduce or inhibit either unimpaired, or if appropriate, mutant protective sequence activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides which are complementary to a protective sequence mRNA. The antisense oligonucleotides will bind to the complementary protective sequence mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required.

A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In one embodiment, oligonucleotides complementary to non-coding regions of the protective sequence of interest could be used in an antisense approach to inhibit translation of endogenous mRNA. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit protective sequence expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the cerebral RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleic acid of the oligonucleotide

differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger, *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre, *et al.*, 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier, *et al.*, 1987, *Nucl. Acids Res.* 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue, *et al.*, 1987, *Nucl. Acids Res.* 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue, *et al.*, 1987, *FEBS Lett.* 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein, *et al.* (1988, *Nucl. Acids Res.* 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin, *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451), *etc.*

While antisense nucleotides complementary to the protective sequence coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

Antisense molecules should be delivered to cells that express the protective sequence *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies which specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

A preferred approach to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs which will form complementary base pairs with the endogenous protective sequence transcripts and thereby

prevent translation of the protective sequence mRNA. For example, a vector can be introduced *e.g.*, such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3'-long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner, *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster, *et al.*, 1982, *Nature* 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct that can be introduced directly into the tissue site. Alternatively, viral vectors can be used that selectively infect the desired tissue, in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of target gene mRNA and, therefore, expression of target gene product. (See, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver, *et al.*, 1990, *Science* 247, 1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, *Current Biology* 4:469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred.

Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions which form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, *Nature*, 334:585-591, which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target gene mRNA, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, *et al.*, 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, *et al.*, 1986, *Nature*, 324:429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences that are present in the target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, *etc.*) and should be delivered to cells that express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies, *et al.*, 1985, *Nature* 317:230-234; Thomas and Capecchi, 1987, *Cell*

51:503-512; Thompson, *et al.*, 1989, *Cell* 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells which express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas and Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (*i.e.*, the target gene promoter and/or enhancers) to form triple helical structures which prevent transcription of the target gene in target cells in the body. (See generally, Helene, 1991, *Anticancer Drug Des.*, 6(6):569-584; Helene, *et al.*, 1992, *Ann. N.Y. Acad. Sci.*, 660:27-36; and Maher, 1992, *Bioassays* 14(12):807-815).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleic acids may be pyrimidine-based, which will result in TAT and $CGC^+$ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen which are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles which the possibility may arise wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules which encode and express target gene polypeptides exhibiting normal target gene activity may, be introduced into cells via gene therapy methods such as those described, below, in Section 5.4.1.3 which do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid-phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

### 5.4.1.3 Gene Replacement Therapy

Protective nucleic acid sequences, described above in Section 5.1, can be utilized for transferring recombinant protective nucleic acid sequences to cells and expressing said sequences in recipient cells. Such techniques can be used, for example, in marking cells or for the treatment of a condition, disorder, or disease involving cell death. Such treatment can be in the form of gene replacement therapy. Specifically, one or more copies of a normal protective sequence or a portion of the protective sequence which directs the production of a protective sequence product exhibiting normal protective sequence function, may be inserted into the appropriate cells within a patient, using vectors which include, but are not limited to adenovirus, adeno-associated virus and retrovirus vectors, in addition to other particles which introduce DNA into cells, such as liposomes.

Because the protective sequence of the invention may be expressed in the brain, such gene replacement therapy techniques should be capable of delivering protective sequences to these cell types within patients. Thus, in one embodiment, techniques which are well known to those of skill in the art (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988) can be used to enable protective sequences to cross the blood-brain barrier readily and to deliver the sequences to cells in the brain. With respect to delivery which is capable of crossing the blood-brain barrier, viral vectors such as, for example, those described above, are preferable.

In another embodiment, techniques for delivery involve direct administration, *e.g.*, by stereotactic delivery of such protective sequences to the site of the cells in which the protective sequences are to be expressed.

Methods for introducing genes for expression in mammalian cells are well known in the field. Generally, for such gene therapy methods, the nucleic acid is directly administered *in vivo* into a target cell or a transgenic mouse that expresses SP-10 promoter operably linked to a reporter gene. This can be accomplished by any methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), by direct injection of naked DNA, by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), by coating with lipids or cell-surface receptors or transfecting agents, by encapsulation in

liposomes, microparticles, or microcapsules, by administering it in linkage to a peptide which is known to enter the nucleus, or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), which can be used to target cell types specifically expressing the receptors. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated April 16, 1992; WO 92/22635 dated December 23, 1992; WO92/20316 dated November 26, 1992; WO93/14188 dated July 22, 1993; WO 93/20221 dated October 14, 1993).

Additional methods which may be utilized to increase the overall level of protective sequence expression and/or gene product activity include using targeted homologous recombination methods, discussed in Section 5.2, above, to modify the expression characteristics of an endogenous protective sequence in a cell or microorganism by inserting a heterologous DNA regulatory element such that the inserted regulatory element is operatively linked with the endogenous protective sequence in question. Targeted homologous recombination can thus be used to activate transcription of an endogenous protective sequence which is "transcriptionally silent", *i.e.*, is not normally expressed or is normally expressed at very low levels, or to enhance the expression of an endogenous protective sequence which is normally expressed.

Further, the overall level of protective sequence expression and/or gene product activity may be increased by the introduction of appropriate protective sequence-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of a condition, disorder, or disease involving cell death. Such cells may be either recombinant or non-recombinant.

Among the cells that can be administered to increase the overall level of protective sequence expression in a patient are normal cells, preferably brain cells, which express the protective sequence. Alternatively, cells, preferably autologous cells, can be engineered to express protective sequences, and may then be introduced into a patient in positions appropriate for the amelioration of the symptoms of a condition, disorder, or disease involving cell death. Alternately, cells which express an unimpaired protective sequence and

which are from a MHC matched individual can be utilized, and may include, for example, brain cells. The expression of the protective sequences is controlled by the appropriate gene regulatory sequences to allow such expression in the necessary cell types. Such gene regulatory sequences are well known to the skilled artisan. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, U.S. Patent No. 5,399,349.

When the cells to be administered are non-autologous cells, they can be administered using well-known techniques that prevent a host immune response against the introduced cells from developing. For example, the cells may be introduced in an encapsulated form that, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Additionally, compounds, such as those identified via techniques such as those described, in Section 5.4.2, which are capable of modulating protective sequences, protective sequence product activity, or their regulatory sequences can be administered using standard techniques which are well known to those of skill in the art. In instances in which the compounds to be administered are to involve an interaction with brain cells, the administration techniques should include well known methods that allow for a crossing of the blood-brain barrier.

#### 5.4.1.4 Detection of Protective Nucleic Acid Molecules

A variety of methods can be employed to screen for the presence of protective sequence-specific mutations or polymorphisms (including polymorphisms flanking protective sequences) and to detect and/or assay levels of protective nucleic acid sequences.

Mutations or polymorphisms within or flanking the protective sequences can be detected by utilizing a number of techniques. Nucleic acid from any nucleated cell can be used as the starting point for such assay techniques, and may be isolated according to standard nucleic acid preparation procedures that are well known to those of skill in the art.

Protective nucleic acid sequences may be used in hybridization or amplification assays of biological samples to detect abnormalities involving protective sequence structure, including point mutations, insertions, deletions, inversions, translocations

and chromosomal rearrangements. Such assays may include, but are not limited to, Southern analyses, single-stranded conformational polymorphism analyses (SSCP) and PCR analyses.

Diagnostic methods for the detection of protective sequence-specific mutations or polymorphisms can involve for example, contacting and incubating nucleic acids obtained from a sample, *e.g.*, derived from a patient sample or other appropriate cellular source with one or more labeled nucleic acid reagents including recombinant DNA molecules, cloned genes or degenerate variants thereof, such as described in Section 5.1, above, under conditions favorable for the specific annealing of these reagents to their complementary sequences within or flanking the protective sequence. The diagnostic methods of the present invention further encompass contacting and incubating nucleic acids for the detection of single nucleotide mutations or polymorphisms of the protective sequence. Preferably, these nucleic acid reagent sequences within the protective sequence are 15 to 30 nucleotides in length.

After incubation, all non-annealed nucleic acids are removed from the reaction. The presence of nucleic acids that have hybridized, if any such molecules exist, is then detected. Using such a detection scheme, the nucleic acid from the cell type or tissue of interest can be immobilized, for example, to a solid support such as a membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-annealed, labeled nucleic acid reagents of the type described in Section 5.1 are easily removed. Detection of the remaining, annealed, labeled nucleic acid reagents is accomplished using standard techniques well known to those skilled in the art. The protective sequences of the invention to which the nucleic acid reagents have annealed can be compared to the annealing pattern expected from a normal protective sequence of the invention in order to determine whether a protective sequence mutation is present.

In a preferred embodiment, protective sequence mutations or polymorphisms can be detected by using a microassay of nucleic acid sequences of the invention immobilized to a substrate or "gene chip" (see, *e.g.* Cronin, et al., 1996, Human Mutation 7:244-255). Alternative diagnostic methods for the detection of protective sequence-specific nucleic acid molecules (or flanking sequences), in patient samples or other appropriate cell sources, may involve their amplification, *e.g.*, by PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), followed by the analysis of the amplified molecules using

techniques well known to those of skill in the art, such as, for example, those listed above. The resulting amplified sequences can be compared to those which would be expected if the nucleic acid being amplified contained only normal copies of the protective sequence in order to determine whether a protective sequence mutation or polymorphism in linkage disequilibrium with a disease-causing allele exists.

Among those nucleic acid sequences that are preferred for such amplification-related diagnostic screening analyses are oligonucleotide primers that amplify exon sequences. The sequences of such oligonucleotide primers are, therefore, preferably derived from cerebral intron sequences so that the entire exon, or coding region, can be analyzed as discussed below. Primer pairs useful for amplification of cerebral exons are preferably derived from adjacent introns. Appropriate primer pairs can be chosen such that each of the cerebral exons present within the gene will be amplified. Primers for the amplification of exons can be routinely designed by one of ordinary skill.

Additional nucleic acid sequences which are preferred for such amplification-related analyses are those which will detect the presence of a polymorphism which differs from the sequence depicted in the Figures. Such polymorphisms include ones that represent mutations associated with a condition, disorder, or disease involving cell death.

Amplification techniques are well known to those of skill in the art and can routinely be utilized in connection with primers such as those described above. In general, hybridization conditions can be as follows: In general, for probes between 14 and 70 nucleotides in length, the melting temperature TM is calculated using the formula: Tm(°C)=81.5+16.6(log[monovalent cations])+0.41(% G+C)-(500/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature is calculated using the equation Tm(°C)=81.5+16.6(log[monovalent cations])+0.41(% G+C)-(0.61% formamide)-(500/N) where N is the length of the probe. Additionally, well-known genotyping techniques can be performed to identify individuals carrying protective sequence mutations. Such techniques include, for example, the use of restriction fragment length polymorphisms (RFLPs), which involve sequence variations in one of the recognition sites for the specific restriction enzyme used.

Further, improved methods for analyzing DNA polymorphisms, which can be utilized for the identification of protective sequence-specific mutations, have been described

which capitalize on the presence of variable numbers of short, tandemly repeated DNA sequences between the restriction enzyme sites. For example, Weber (U.S. Pat. No. 5,075,217) describes a DNA marker based on length polymorphisms in blocks of (dC-dA)n-(dG-dT)n short tandem repeats. The average separation of (dC-dA)n-(dG-dT)n blocks is estimated to be 30,000-60,000 bp. Markers which are so closely spaced exhibit a high frequency of co-inheritance, and are extremely useful in the identification of genetic mutations, such as, for example, mutations within the protective sequence of the invention, and the diagnosis of diseases and disorders related to mutations of the protective sequences of the invention.

Also, Caskey *et al.* (U.S. Pat.No. 5,364,759) describe a DNA profiling assay for detecting short tri- and tetra nucleotide repeat sequences. The process includes extracting the DNA of interest, amplifying the extracted DNA and labeling the repeat sequences to form a genotypic map of the individual's DNA.

Other methods well known in the art may be used to identify single nucleotide polymorphisms (SNPs), including biallelic SNPs or biallelic markers which have two alleles, both of which are present at a fairly high frequency in a population. Conventional techniques for detecting SNPs include, *e.g.*, conventional dot blot analysis, single stranded conformational polymorphism (SSCP) analysis (see, *e.g.*, Orita *et al.*, 1989, *Proc. Natl. Acad. Sci. USA 86*:2766-2770), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, mismatch cleavage detection and other routine techniques well known in the art (see, *e.g.*, Sheffield *et al.*, 1989, *Proc. Natl. Acad. Sci. 86*:5855-5892; Grompe, 1993, *Nature Genetics 5*:111-117). Alternative, preferred methods of detecting and mapping SNPs involve microsequencing techniques wherein an SNP site in a target DNA is detecting by a single nucleotide primer extension reaction (see, *e.g.*, Goelet *et al.*, PCT Publication No. WO92/15712; Mundy, U.S. Patent No. 4,656,127; Vary and Diamond, U.S. Patent No. 4,851,331; Cohen *et al.*, PCT Publication No. WO91/02087; Chee *et al.*, PCT Publication No. WO95/11995; Landegren *et al.*, 1988, *Science 241*:1077-1080; Nicerson *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A. 87*:8923-8927; Pastinen *et al.*,1997, *Genome Res. 7*:606-614; Pastinen *et al.*, 1996, *Clin. Chem. 42*:1391-1397; Jalanko *et al.*, 1992, *Clin. Chem. 38*:39-43; Shumaker *et al.*, 1996, *Hum. Mutation 7*:346-354; Caskey *et al.*, PCT Publication No. WO 95/00669).

The level of protective sequence expression also can be assayed. For example, RNA from a cell type or tissue known, or suspected, to express the protective sequence, such as brain, may be isolated and tested utilizing hybridization or PCR techniques such as are described, above. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the protective sequence. Such analyses may reveal both quantitative and qualitative aspects of the expression pattern of the protective sequence, including activation or inactivation of protective sequence expression.

In one embodiment of such a detection scheme, a cDNA molecule is synthesized from an RNA molecule of interest (*e.g.*, by reverse transcription of the RNA molecule into cDNA). A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the protective sequence nucleic acid reagents described in Section 5.1. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified product, the nucleic acid amplification may be performed using radioactively or non-radioactively labeled nucleotides. Alternatively, enough amplified product may be made such that the product may be visualized by standard ethidium bromide staining or by utilizing any other suitable nucleic acid staining method.

Additionally, it is possible to perform such protective sequence expression assays "*in situ*", *i.e.*, directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents such as those described in Section 5.1 may be used as probes and/or primers for such *in situ* procedures (see, for example, Nuovo, G.J., 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard Northern blot analysis can be performed to determine the level of mRNA expression of the protective sequence.

### 5.4.1.5 Detection of Protective Sequence Products

Protective sequence products of the invention, including both wild-type and mutant protective sequence products, conserved variants and polypeptide fragments thereof, which are discussed, above, in Section 5.2, may be detected using antibodies which are directed against such gene products. Such antibodies, which are discussed in Section 5.3, above, may thereby be used as diagnostics and prognostics for a condition, disorder, or disease involving cell death. Such methods may be used to detect abnormalities in the level of protective sequence expression or of protective sequence product synthesis, or abnormalities in the structure, temporal expression and/or physical location of protective sequence product. The antibodies and immunoassay methods described herein have, for example, important *in vitro* applications in assessing the efficacy of treatments for conditions, disorders, or diseases involving cell death. Antibodies, or fragments of antibodies, such as those described below, may be used to screen potentially therapeutic compounds *in vitro* to determine their effects on protective sequence expression and protective sequence product production. The compounds which have beneficial effects on conditions, disorders, or diseases involving cell death can thereby be identified, and a therapeutically effective dose determined.

*In vitro* immunoassays may also be used, for example, to assess the efficacy of cell-based gene therapy for a condition, disorder, or disease involving cell death. Antibodies directed against protective sequence products may be used *in vitro* to determine, for example, the level of protective sequence expression achieved in cells genetically engineered to produce the protective sequence product. In the case of intracellular protective sequence products, such an assessment is done, preferably, using cell lysates or extracts. Such analysis will allow for a determination of the number of transformed cells necessary to achieve therapeutic efficacy *in vivo*, as well as optimization of the gene replacement protocol.

The tissue or cell type to be analyzed generally will include those that are known, or suspected, to express the protective sequence. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to

be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the protective sequence.

Preferred diagnostic methods for the detection of protective sequence products, conserved variants or peptide fragments thereof, may involve, for example, immunoassays wherein the protective sequence products or conserved variants or peptide fragments are detected by their interaction with an anti-protective sequence product-specific antibody.

For example, antibodies, or fragments of antibodies, such as those described, above, in Section 5.3, may be used to, quantitatively or qualitatively, detect the presence of protective sequence products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below, this Section) coupled with light microscopic, flow cytometric or fluorimetric detection. Such techniques are especially preferred for protective sequence products that are expressed on the cell surface.

The antibodies (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of protective sequence products, conserved variants or peptide fragments thereof. *In situ* detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody which binds to a protective sequence polypeptide. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the protective sequence product, conserved variants or peptide fragments, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily recognize that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve *in situ* detection of a protective sequence product.

Immunoassays for protective sequence products, conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells or lysates of cells in the presence of a detectably labeled antibody capable of identifying the protective sequence product, conserved variants or peptide

fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier, such as nitrocellulose, which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled protective sequence product specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on the solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

One of the ways in which the protective sequence product-specific antibody can be detectably labeled is by linking the same to an enzyme, such as for use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller, A. *et al.*, 1978, *J. Clin. Pathol.* 31:507-520; Butler, J.E., 1981, *Meth. Enzymol.* 73:482-523; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. *et al.*, (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme that is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means.

Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, a-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, b-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods that employ a chromogenic substrate for the enzyme. Detection also may be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may be accomplished also using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect protective sequence products through the use of a radioimmunoassay (RIA) (*see*, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as $^{152}$Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

### 5.4.2 Screening Assays for Compounds which Interact with Protective Sequence Products or Modulate Protective Sequence Activity

The following assays are designed to identify compounds which bind to a protective sequence product, compounds which bind to proteins, or portions of proteins which interact with a protective sequence product, compounds which modulate, *e.g.*, interfere with, the interaction of a protective sequence product with proteins and compounds which modulate the activity of the protective sequence (*i.e.*, modulate the level of protective sequence expression and/or modulate the level of protective sequence product activity). Assays may additionally be utilized which identify compounds which bind to protective sequence regulatory sequences (*e.g.*, promoter sequences; see *e.g.*, Platt, 1994, J. Biol. Chem. 269, 28558-28562), and which can modulate the level of protective sequence expression. Such compounds may include, but are not limited to, small organic molecules, such as ones which are able to cross the blood-brain barrier, gain to and/or entry into an appropriate cell and affect expression of the protective sequence or some other gene involved in a protective sequence regulatory pathway.

Methods for the identification of such proteins are described, below, in Section 5.4.2.2. Such proteins may be involved in the control and/or regulation of functions related to cell death. Further, among these compounds are compounds which affect the level of protective sequence expression and/or protective sequence product activity and which can be used in the therapeutic treatment of conditions, disorders, or diseases involving cell death as described, below, in Section 5.4.2.3.

Compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to, Ig-tailed fusion peptides, and members of random peptide libraries; (see, *e.g.*, Lam, *et al.*, 1991, *Nature* 354:82-84; Houghten, *et al.*,

1991, *Nature* 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang, *et al.*, 1993, *Cell* 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, $F(ab')_2$ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Such compounds may further comprise compounds, in particular drugs or members of classes or families of drugs, known to ameliorate the symptoms of a condition, disorder, or disease involving cell death.

Such compounds include families of antidepressants such as lithium salts, carbamazepine, valproic acid, lysergic acid diethylamide (LSD), *p*-chlorophenylalanine, *p*-propyldopacetamide dithiocarbamate derivatives *e.g.*, FLA 63; anti-anxiety drugs, *e.g.*, diazepam; monoamine oxidase (MAO) inhibitors, *e.g.*, iproniazid, clorgyline, phenelzine and isocarboxazid; biogenic amine uptake blockers, *e.g.*, tricyclic antidepressants such as desipramine, imipramine and amitriptyline; serotonin reuptake inhibitors *e.g.*, fluoxetine; antipsychotic drugs such as phenothiazine derivatives (*e.g.*, chlorpromazine (thorazine) and trifluopromazine)), butyrophenones (*e.g.*, haloperidol (Haldol)), thioxanthene derivatives (*e.g.*, chlorprothixene), and dibenzodiazepines (*e.g.*, clozapine); benzodiazepines; dopaminergic agonists and antagonists *e.g.*, L-DOPA, cocaine, amphetamine, a-methyl-tyrosine, reserpine, tetrabenazine, benzotropine, pargyline; noradrenergic agonists and antagonists *e.g.*, clonidine, phenoxybenzamine, phentolamine, tropolone.

Compounds identified via assays such as those described herein may be useful, for example, in elaborating the biological function of protective sequence products and for ameliorating conditions, disorders, or diseases involving cell death. Assays for testing the effectiveness of compounds identified by, for example, techniques such as those described in Sections 5.4.2.1 - 5.4.2.3, are discussed, below, in Section 5.4.2.4.

### 5.4.2.1 *In Vitro* Screening Assays for Compounds which Bind to Protective Sequence Products

*In vitro* systems may be designed to identify compounds capable of binding the protective sequence products of the invention. Compounds identified may be useful, for example, in modulating the activity of unimpaired and/or mutant protective sequence products, may be useful in elaborating the biological function of the protective sequence product, may be utilized in screens for identifying compounds which disrupt normal protective sequence product interactions or may in themselves disrupt such interactions.

The principle of the assays used to identify compounds which bind to the protective sequence product involves preparing a reaction mixture of the protective sequence product and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay involves anchoring a protective sequence product or a test substance onto a solid support and detecting protective sequence product/test compound complexes formed on the solid support at the end of the reaction. In one embodiment of such a method, the protective sequence product may be anchored onto a solid support, and the test compound, which is not anchored, may be labeled, either directly or indirectly.

In practice, microtiter plates are conveniently utilized as the solid support. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-

immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously non-immobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for the protective sequence product or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

#### 5.4.2.2 Assays for Proteins which Interact with Protective Sequence Products

Any method suitable for detecting protein-protein interactions may be employed for identifying protective sequence product-protein interactions.

Among the traditional methods that may be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns. Utilizing procedures such as these allows for the identification of proteins, including intracellular proteins, which interact with protective sequence products. Once isolated, such a protein can be identified and can be used in conjunction with standard techniques, to identify proteins it interacts with. For example, at least a portion of the amino acid sequence of a protein which interacts with the protective sequence product can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique (see, *e.g.*, Creighton, 1983, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for gene sequences encoding such proteins. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well known. (See, *e.g.*, Ausubel, *supra*, and 1990, "PCR

Protocols: A Guide to Methods and Applications," Innis, *et al.*, eds. Academic Press, Inc., New York).

Additionally, methods may be employed which result in the simultaneous identification of genes that encode a protein that interacts with a protective sequence product. These methods include, for example, probing expression libraries with labeled protective sequence product, using the protective sequence product in a manner similar to the well-known technique of antibody probing of lgt11 libraries.

One method that detects protein interactions *in vivo*, the two-hybrid system, is described in detail for illustration only and not by way of limitation. One version of this system has been described (Chien, *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88:9578-9582) and is commercially available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed which encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the protective sequence product and the other consists of the transcription activator protein's activation domain fused to an unknown protein which is encoded by a cDNA which has been recombined into this plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (*e.g.*, HBS or *lacZ*) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene: the DNA-binding domain hybrid cannot because it does not provide activation function and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodologies may be used to screen activation domain libraries for proteins that interact with the "bait" gene product. By way of example, and not by way of limitation, protective sequence products of the invention may be used as the bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of a bait protective sequence product fused to the DNA-binding domain are co-transformed into a yeast reporter strain, and the resulting transformants are screened for those which express the

reporter gene. For example, a bait protective sequence, such as the open reading frame of the gene, can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line, from which proteins which interact with bait protective sequence products are to be detected, can be made using methods routinely practiced in the art. According to the particular system described herein, for example, the cDNA fragments can be inserted into a vector such that they are translationally fused to the transcriptional activation domain of GAL4. Such a library can be co-transformed along with the bait protective sequence-GAL4 fusion plasmid into a yeast strain that contains a lacZ gene driven by a promoter that contains GAL4 activation sequence. A cDNA encoded protein, fused to a GAL4 transcriptional activation domain that interacts with bait protective sequence product will reconstitute an active GAL4 protein and thereby drive expression of the HIS3 gene. Colonies that express HIS3 can be detected by their growth on petri dishes containing semi-solid agar based media lacking histidine. The cDNA can then be purified from these strains, and used to produce and isolate the bait protective sequence product-interacting protein using techniques routinely practiced in the art.

#### 5.4.2.3 Assays for Compounds which Interfere with or Potentiate Protective Sequence Products Macromolecule Interaction

The protective sequence products may, *in vivo*, interact with one or more macromolecules, including intracellular macromolecules, such as proteins. Such macromolecules may include, but are not limited to, nucleic acid molecules and those proteins identified via methods such as those described, above, in Sections 5.4.2.1 - 5.4.2.2. For purposes of this discussion, the macromolecules are referred to herein as "binding partners". Compounds that disrupt protective sequence product binding to a binding partner may be useful in regulating the activity of the protective sequence product, especially mutant protective sequence products. Such compounds may include, but are not limited to molecules such as peptides, and the like, as described, for example, in Section 5.4.2.1 above.

The basic principle of an assay system used to identify compounds which interfere with or potentiate the interaction between the protective sequence product and a binding partner or partners involves preparing a reaction mixture containing the protective sequence product and the binding partner under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of protective sequence product and its binding partner. Control reaction mixtures are incubated without the test compound or with a compound that is known not to block complex formation. The formation of any complexes between the protective sequence product and the binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the protective sequence product and the binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal protective sequence product also may be compared to complex formation within reaction mixtures containing the test compound and a mutant protective sequence product. This comparison may be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal protective sequence product.

In order to test a compound for potentiating activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of protective sequence product and its binding partner. Control reaction mixtures are incubated without the test compound or with a compound that is known not to block complex formation. The formation of any complexes between the protective sequence product and the binding partner is then detected. Increased formation of a complex in the reaction mixture containing the test compound, but not in the control reaction, indicates that the compound enhances and therefore potentiates the interaction of the protective sequence product and the binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal protective sequence product may also be compared to complex formation within reaction mixtures containing the test compound and a mutant protective

sequence product. This comparison may be important in those cases wherein it is desirable to identify compounds that enhance interactions of mutant but not normal protective sequence product.

In alternative embodiments, the above assays may be performed using a reaction mixture containing the protective sequence product, a binding partner and a third compound which disrupts or enhances protective sequence product binding to the binding partner. The reaction mixture is prepared and incubated in the presence and absence of the test compound, as described above, and the formation of any complexes between the protective sequence product and the binding partner is detected. In this embodiment, the formation of a complex in the reaction mixture containing the test compound, but not in the control reaction, indicates that the test compound interferes with the ability of the second compound to disrupt protective sequence product binding to its binding partner.

The assays for compounds that interfere with or potentiate the interaction of the protective sequence products and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the protective sequence product or the binding partner onto a solid support and detecting complexes formed on the solid support at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds which interfere with or potentiate the interaction between the protective sequence products and the binding partners, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior to or simultaneously with the protective sequence product and interactive intracellular binding partner. Alternatively, test compounds which disrupt preformed complexes, *e.g.*, compounds with higher binding constants which displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the protective sequence product or the interactive binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized.

The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the protective sequence product or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex formation or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the protective sequence product and the interactive binding partner is prepared in which either the protective sequence product or its binding partners is labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above

background. In this way, test substances that disrupt protective sequence product/binding partner interaction can be identified.

In another embodiment of the invention, these same techniques can be employed using peptide fragments which correspond to the binding domains of the protective sequence product and/or the binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the segments is engineered to express peptide fragments of the protein, it can then be tested for binding activity and purified or synthesized.

For example, and not by way of limitation, a protective sequence product can be anchored to a solid material as described, above, in this Section by making a GST-1 fusion protein and allowing it to bind to glutathione agarose beads. The binding partner can be labeled with a radioactive isotope, such as $^{35}S$, and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added to the anchored GST-1 fusion protein and allowed to bind. After washing away unbound peptides, labeled bound material, representing the binding partner binding domain, can be eluted, purified and analyzed for amino acid sequence by well-known methods. Peptides so identified can be produced synthetically or produced using recombinant DNA technology.

#### 5.4.2.4 <u>Assays for the Identification of Compounds which Modulate Conditions, Disorders, or Diseases Involving Cell Death</u>

Compounds, including, but not limited to, binding compounds identified via assay techniques such as those described, above, in Sections 5.4.2.1 - 5.4.2.3, can be tested for the ability to ameliorate symptoms of a condition, disorder, or disease involving cell death.

It should be noted that the assays described herein can be used to identify compounds which affect activity by either affecting protective sequence expression or by affecting the level of protective sequence product activity. For example, compounds may be identified which are involved in another step in the pathway in which the protective sequence and/or protective sequence product is involved, such as, for example, a step which is either "upstream" or "downstream" of the step in the pathway mediated by the protective sequence. Such compounds may, by affecting this same pathway, modulate the effect on the development of conditions, disorders, or diseases involving cell death. Such compounds can be used as part of a therapeutic method for the treatment of the condition, disorder, or disease.

Described below are cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate symptoms of a condition, disorder, or disease involving cell death.

First, cell-based systems can be used to identify compounds which may act to ameliorate symptoms of a condition, disorder, or disease, including, but not limited to, those described in Section 5.4.1.1. Such cell systems can include, for example, recombinant or non-recombinant cell, such as cell lines, which express the protective sequence of interest.

In utilizing such cell systems, cells which express the protective sequence of interest may be exposed to a compound suspected of exhibiting an ability to ameliorate symptoms of a condition, disorder, or disease involving cell death at a sufficient concentration and for a sufficient time to elicit such an amelioration of such symptoms in the exposed cells. After exposure, the cells can be assayed to measure alterations in the expression of the protective sequence, *e.g.*, by assaying cell lysates for cerebral mRNA transcripts (*e.g.*, by Northern analysis) or for protective sequence products expressed by the cell; compounds which modulate expression of the protective sequence are good candidates

as therapeutics.

In addition, animal-based systems or models for a condition, disorder, or disease involving cell death, for example, transgenic mice containing a human or altered form of a protective sequence, may be used to identify compounds capable of ameliorating symptoms of the condition, disorder, or disease. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies and interventions. For example, animal models may be exposed to a compound suspected of exhibiting an ability to ameliorate symptoms, at a sufficient concentration and for a sufficient time to elicit such an amelioration of symptoms of a condition, disorder, or disease involving cell death. The response of the animals to the exposure may be monitored by assessing the reversal of the symptoms of the condition, disorder, or disease.

With regard to intervention, any treatments that reverse any aspect of symptoms of a condition, disorder, or disease involving cell death, should be considered as candidates for human therapeutic intervention in such conditions, disorders, or diseases. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 5.5.1, below.

#### 5.4.3 Additional Uses for the Protective Sequences, Protective Sequence Products, or Their Regulatory Elements

In addition to the uses described above, the polynucleotides of the present invention can be used for various other purposes. For example, they can be used to express recombinant protein for analysis, characterization or therapeutic use; as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic conditions, disorders, or diseases; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response.

The proteins provided by the present invention can similarly be used to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

## 5.5 Pharmaceutical Preparations and Methods of Administration

The compounds which are determined to affect protective sequence expression or gene product activity can be administered to a patient at therapeutically effective doses to treat or ameliorate a condition, disorder, or disease involving cell death or modulate a cell death-related process described herein. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of such a condition, disorder, or disease.

### 5.5.1 Effective Dose

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio $LD_{50}/ED_{50}$.

Compounds that exhibit large therapeutic indices are preferred. While compounds which exhibit toxic side effects may be used, care should be taken to design a delivery system which targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range which includes the $IC_{50}$ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of antibody, protein, or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or condition, disorder, or disease, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in

dosage may result and become apparent from the results of diagnostic assays as described herein.

### 5.5.2 Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral rectal or topical administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In certain embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

For topical application, the compounds may be combined with a carrier so that an effective dosage is delivered, based on the desired activity.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

# 6 EXAMPLE: SEQUENCE AND CHARACTERIZATION OF PROTECTIVE SEQUENCES

In the example presented herein, the sequence and characterization of the protective sequences are provided.

## 6.1 Materials and Methods

### 6.1.1 Preparation of DNA

A human fetal brain cDNA library (Gibco), in which individual clones were inserted into the NotI-SalI site of the pCMV·SPORT2 vector, was diluted 200,000 fold in LB broth (DIFCO Laboratories) containing 0.2 mg/ml ampicillin (Sigma). The diluted library (100-140 $\mu$l) was then plated and grown on LB agar (DIFCO Laboratories) bioassay plates with 0.2 mg/ml ampicillin. Plates were incubated at 37°C for 24 hours. Single colonies were then used to inoculate deep-well blocks containing 1.5 ml LB broth containing 0.2 mg/ml ampicillin. Inoculated cultures were incubated at 37°C with agitation at 150-200 rpm for 18-24 hours. Replicate plates were created from the cultures by adding 20 $\mu$l of culture to 80 $\mu$l of LB broth containing 18% glycerol and 0.2 mg/ml ampicillin and stored at -80°C. Remaining bacterial cells were centrifuged at 1000 x g for 6 minutes to collect the cells at the bottom. Following centrifugation, the broth was decanted off of the bacterial pellet and the pellet resuspended and then stored in 100 $\mu$l of Cell Resuspension Solution (Promega) at 4°C for up to one week.

Plasmid DNA was extracted using Promega MagneSil kits with a modified protocol. The pelleted bacteria were re-suspended and 50 $\mu$l was transferred into a round bottom plate that rests on a magnet. Cell Lysis Solution (50 $\mu$l) was added and the plate was incubated at room temperature without agitation for 30 seconds. Following lysis, 70 $\mu$l of a Neutralization Solution/MagneSil Paramagnetic Particles mixture (pre-mixed at a ratio of

6:1) was added. The reaction was mixed by pipetting and incubated at room temperature without agitation for 5 minutes to allow the magnetic particles to be drawn to the magnet. The supernatant containing plasmid DNA was then transferred to a new plate and stored at -20°C.

Individual clones were chosen for their ability to delay or prevent cell death when introduced into a cell predisposed to undergoing cell death, relative to a corresponding cell into which no exogenous protective sequence had been introduced.

### 6.1.2 Sequence Characterization of the DNA

The cDNA inserts of the clonally pure plasmids which are selected for their ability to protect cells from cell death when introduced into cells predisposed to undergo cell death are sequenced using the ABI Big Dye terminator Cycle Sequencing Ready Reaction Kit and subsequently analyzed on the ABI310 capillary sequencing machine (PE Biosystems, Foster City, CA).

Briefly, 0.5 mg of plasmid DNA is mixed with 3.2 pmole of either the M13 forward (5'-TGTAAAACGACGGCCAGT-3'; SEQ ID NO:465) or the M13 reverse (5'-CAGGAAACAGCTATGACC-3'; SEQ ID NO:466) sequencing primer and 8 ml of the terminator ready reaction mix in a total volume of 20 ml. The cycle sequencing reaction is carried out in a thermocycler (PCR machine) using standard methods known by those skilled in the art. The extension products from the sequencing reaction are purified by precipitation using isopropanol. 80 ml of 75% isopropanol is added to the sample and after thorough mixing, the sample is incubated at room temperature (25°C) for 20 minutes. The sample is then centrifuged at 12,000 x g for 20 minutes at room temperature. The supernatant is removed and the pellet is rinsed once by addition of 250 ml of 75% isopropanol followed by centrifugation as above for 5 minutes. The supernatant is removed and the sample air-dried for 10 minutes. The sample is then resuspended in 20 ml of TSR (template suppression reagent) and denatured by heating at 94°C for 2 minutes and rapidly cooling on ice. The subsequent electrophoresis and analysis is carried out on the ABI310 sequencer according to the manufacturer's protocol. The entire cDNA clone is similarly sequenced by the use of sequence specific internal primers as required.

### 6.1.3 Sequence Comparison

The sequence data for the protective cDNA clones is compared using the BLAST 2.0 algorithm (Altschul, SF *et al.*, 1997, Nuc. Acids Res. 25:3389) against known sequences in the GeneBank sequence database maintained by NCBI (National Center for Biotechnology Information). This program uses the two-hit method to find homology within the database. The BLAST nucleotide searches are performed with the "BLAST N" program (wordlength = 11) to obtain nucleic acids homologous to nucleic acid molecules of the invention. BLAST protein searches of potential ORFs are performed with the "BLAST P" program (wordlength = 3) to obtain amino acid sequences homologous to the ORFs of the invention.

### 6.1.4 Immuno-Cytochemistry Protocol for the Characterization of Protected Cells

Transfected tissue is immersed in freshly prepared 2.5% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for two hours to fix the tissue. PFA is removed by aspiration and the fixed tissue washed consecutively four times in PBS for 15 minutes, changing the PBS solution between each wash. Upon removal of the final PBS wash, the tissue is immersed in a blocking solution consisting of 10% goat serum, 2% bovine serum albumin (BSA), and 0.25% Triton X-100 for a duration of two hours.

After removal of the blocking solution, the tissue is immersed in a primary antibody solution, freshly prepared by adding rabbit anti-GFP polyclonal (1:2000 ul) into blocking solution, for an incubation period of twelve hours at 4°C.

After removal of the primary antibody solution, the tissue is washed consecutively four times in PBS for 10 minutes, changing the PBS solution between each wash. An anti-rabbit, flourescently conjugated secondary antibody, diluted in PBS at a concentration of 1:500, is then added to the tissue and allowed to incubate at room temperature for four hours. The secondary antibody solution is removed by aspiration and the tissue washed consecutively four times in PBS for 15 minutes, changing the PBS solution between each wash. After the final wash is removed, the tissue is mounted on glass slides and dried at 37°C for thirty minutes. A three-minute xylene incubation is performed before the addition of coverslips to preserve the slices.

## 6.2 Results

The following protective sequences, which were obtained using the methods described in Section 6.1, were chosen based on their ability to prevent, delay, or rescue cells predisposed to undergo cell death, relative to a corresponding cell into which no exogenous protective sequence had been introduced.

### 6.2.1 Protective sequence CNI-00718

Protective sequence CNI-00718 (SEQ. ID NO:1) is a completely novel sequence which comprises 1794 nucleotides. Twenty-eight (28) potential ORFs have been identified within the protective sequence and are depicted in Table 2. The longest ORF is 112 amino acids. BLAST sequence comparison analysis of CNI-00718 against known nucleotide and protein sequences in the GenBank database reveals no significant homology at either the nucleotide or the amino acid level. As shown in Figure 3F, CNI-00718 caused about a 20-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.2 Protective sequence CNI-00722

Protective sequence CNI-00722 (SEQ. ID NO:58) comprises 810 nucleotides. Twelve (12) potential ORFs have been identified within the protective sequence and are depicted in Table 3. The longest ORF of the cDNA encodes 44 amino acids. BLAST sequence comparison analysis of CNI-00722 against known nucleic acids in the GenBank database reveals homology with the sequence encoding the human chromosome 16 BAC clone CIT987-SKA-113A6 (ACC. No. AC002299). At the nucleotide level, the overall percent homology between CNI-00722 and CIT987-SKA-113A6 is 99.6% (783/785 bases). CIT987-SKA-113A6 is an unidentified DNA. As shown in Figure 3F, CNI-00722 caused about a 21-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.3 Protective sequence CNI-00725

Protective sequence CNI-00725 (SEQ. ID NO:83) comprises 920 nucleotides. Eleven (11) potential ORFs have been identified within the protective sequence and are depicted in Table 4. BLAST sequence comparison analysis of CNI-00725 against known nucleic acids in the GenBank database reveals a 97% identity (870/897 bases) with a human mitochondrial sequence encoding the 16S rRNA and tRNA for the amino acid Leucine (ACC. No. V00710). However, most of the homology (95%) is with the 16S rRNA sequence. As shown in Figure 3F, CNI-00725 caused about a 14-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.4 Protective sequence CNI-00726

Protective sequence CNI-00726 (SEQ. ID NO:106) comprises 2144 nucleotides. Twenty-six (26) potential ORFs have been identified within the protective sequence and are depicted in Table 5. The longest ORF of CNI-00726 encodes 147 amino acids. BLAST sequence comparison analysis of CNI-00726 against known nucleic acids in the GenBank database reveals a 99.7% identity (1820/1825 bases) with the human ubiquitin-conjugating enzyme variant 1, UBE2V1 (ACC No. NM_003349); a 99.6% identity (1820/1826 bases) with the human DNA-binding protein CROC-1A (ACC No. U39360); and a 72.5% identity (401/553 bases) with the human MMS2 protein (ACC No. AF049140). At the protein level, CNI-00726 has a 100% identity with the 80-221 amino acid region of UBE2VI; a 97% identity (136/140 amino acids) with the 31-170 amino acid region of CROC-1A; and a 90% identity (132/147 amino acids) with the human MMS2 protein. The enzyme UBE2V1 may be involved in controlling differentiation by affecting the distribution of cells in different phases during the cell cycle (Sancho, *et al.* 1998, *Mol. Cell. Biol.* 18: 576-89). The protein CROC-1A is capable of transcriptionally activating the FOS promoter (Rothofsky & Lin, 1997, *Gene* 195: 141-9; Lin & Rothofsky, U.S. Patent No. 5,736,331). As shown in Figure 3F, CNI-00726 caused about a 19-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

#### 6.2.5 Protective sequence CNI-00727

Protective sequence CNI-00727 (SEQ. ID NO:159) is a completely novel sequence which comprises 1293 nucleotides. Nineteen (19) potential ORFs have been identified within the protective sequence and are depicted in Table 6. The longest ORF is 54 amino acids. BLAST sequence comparison analysis of CNI-00727 against known nucleotide and protein sequences in the GenBank database reveals no significant homology at either the nucleotide or the amino acid level. As shown in Figure 3F, CNI-00727 caused about a 17-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

#### 6.2.6 Protective sequence CNI-00728

Protective sequence CNI-00728 (SEQ. ID NO:198) comprises 1466 nucleotides. Twenty-four (24) potential ORFs have been identified within the protective sequence and are depicted in Table 7. The longest ORF is 59 amino acids. BLAST sequence comparison analysis of CNI-00728 against known nucleic acids in the GenBank database reveals a 99.9% identity (1342/1343 bases) with the 3' untranslated region of human sorting nexin 10 mRNA (ACC. No. AF121860). As shown in Figure 3F, CNI-00728 caused about a 10-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

#### 6.2.7 Protective sequence CNI-00729

Protective sequence CNI-00729 (SEQ. ID NO:247) comprises 1659 nucleotides. Twenty-two (22) potential ORFs have been identified within the protective sequence and are depicted in Table 8. BLAST sequence comparison analysis of CNI-00729 against known nucleic acids in the GenBank database reveals a 99.9% identity (1611/1612 bases) with a human actin binding protein, p57 (ACC No. D44497); a 99.9% identity (1561/1562 bp) with human coronin (ACC No. X89109); and a 99.7% identity (1585/1589 bp) with human coronin-like protein, HCORO1 (ACC No. U34690). At the amino acid level, CNI-00729 is identical to human actin protein, p57; identical to human coronin; and 99% identical (459/461 aa) with human coronin-like protein (Suzuki, Jpn. Kokai Tokkyo Koho Patent No. 96119996). The p57 protein is an actin-binding protein and a member of the

coronin family of proteins. The coronins are proteins involved in cell locomotion, cytokinesis, and actin-mediated cellular processes such as phagocytosis (deHostos, 1999, *Trends Cell Biol.* 9: 345-50). As shown in Figure 3F, CNI-00729 caused about a 13-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.8 Protective sequence CNI-00730

Protective sequence CNI-00730 (SEQ. ID NO:292) comprises 722 nucleotides. Nine (9) potential ORFs have been identified within the protective sequence and are depicted in Table 9. The longest ORF of the cDNA encodes 142 amino acids. BLAST sequence comparison analysis of CNI-00730 against known nucleic acids in the GenBank database reveals homology with the sequence encoding human mitochondrial ATP synthase, F0 complex, subunit 9 (ACC. No. NM_001689). At the nucleotide level, the overall percent homology between CNI-00730 and human mitochondrial ATP synthase, F0 complex, subunit 9 is 99.4% (651/655 bp). At the amino acid level, the CNI-00730 and human mitochondrial ATP synthase, F0 complex, subunit 9 proteins are identical. There are three reported genes (P1, P2, and P3) that encode identical forms of mature human mitochondrial ATP synthase, F0 complex, subunit 9; CNI-00730 is homologous to the P3 gene (Yan, *et al.* 1994, *Genomics* 24: 375-7). Subunit 9 accumulates in the lysosomes of individuals affected with the juvenile and late-infantile forms of neuronal ceroid lipofuscinosis (Batten disease) (Tanner, *et al.*, 1997, *Biochim. Biophys. Acta* 1361: 251-62). As shown in Figure 3F, CNI-00730 caused about a 14-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.9 Protective sequence CNI-00731

Protective sequence CNI-00731 (SEQ. ID NO:311) comprises 364 nucleotides. Seven (7) potential ORFs have been identified within the protective sequence and are depicted in Table 10. The longest ORF is 32 amino acids. BLAST sequence comparison analysis of CNI-00731 against known nucleic acids in the GenBank database reveals a 98.5% identity (322/326 bases) with the 3' untranslated region of human interferon-

induced cellular resistance mediator protein (MxA) mRNA (ACC. No. M30817). As shown in Figure 3F, CNI-00731 caused about an 11-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

### 6.2.10 Protective sequence CNI-00732

Protective sequence CNI-00732 (SEQ. ID NO:326) comprises 1046 nucleotides. Eight (8) potential ORFs have been identified within the protective sequence and are depicted in Table 11. The longest ORF is 50 amino acids. BLAST sequence comparison analysis of CNI-00732 against known nucleic acids in the GenBank database reveals a 94% identity (949/1013 bases) with a human mitochondrial sequence encoding the 12S rRNA and tRNA for the amino acid Valine (ACC. No. V00710). However, most of the homology (97%) is with the 12S rRNA sequence. As shown in Figure 3F, CNI-00732 caused about a 12-fold increase in the number of surviving neurons in stroked rat cortical brain slices compared to negative control slices which were exposed to EGFP with no protective sequence.

## 7 DEPOSIT OF DNA

The following DNA clones were deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and comply with the criteria set forth in 37 C.F.R. § 1.801-1.809 regarding availability and permanency of deposits. The deposits were made on the date indicated and assigned the indicated accession number:

| Microorganism Deposit | ATCC Deposit No. | Date of Deposit |
|---|---|---|
| CNI-NPP2-CP10 | PTA-1492 | March 16, 2000 |

CNI-NPP2-CP10 represents a composite deposit of a mixture of ten (10) DNA clones. To distinguish and isolate each of the individual DNA, a sample of the DNA preparation can be digested with *Not* I and *Sal* I, and the resulting products can be separated

by standard gel electrophoresis techniques using a 1% agarose gel in TAE buffer. Liberated inserts are of the following approximate sizes:

| | | |
|---|---|---|
| 1: | CNI-00718 | 1794 bp |
| 2: | CNI-00722 | 810 bp |
| 3: | CNI-00725 | 920 bp |
| 4: | CNI-00726 | 2144 bp |
| 5: | CNI-00727 | 1293 bp |
| 6: | CNI-00728 | 1466 bp |
| 7: | CNI-00729 | 1659 bp |
| 8: | CNI-00730 | 722 bp |
| 9: | CNI-00731 | 364 bp |
| 10: | CNI-00732 | 1046 bp |

## 8 REFERENCES CITED

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

2. An isolated nucleic acid molecule comprising:

(a) a nucleic acid sequence shown in Figures 4(A-AB);

(b) a nucleic acid sequence shown in Figures 5(A-L);

(c) a nucleic acid sequence shown in Figures 6(A-K);

(d) a nucleic acid sequence shown in Figures 7(A-Z);

(e) a nucleic acid sequence shown in Figures 8(A-S);

(f) a nucleic acid sequence shown in Figures 9(A-X);

(g) a nucleic acid sequence shown in Figures 10(A-V);

(h) a nucleic acid sequence shown in Figures 11(A-I);

(i) a nucleic acid sequence shown in Figures 12(A-G);

(j) a nucleic acid sequence shown in Figure 13 (A-H); or

(k) a nucleic acid sequence shown in Figures 1(A-J).

3. An isolated nucleic acid molecule comprising a complement of the nucleic acid molecule of any one of Claims 1 and 2.

4. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of Claim 3 under highly stringent conditions.

5. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of Claim 3 under moderately stringent conditions.

6. The isolated nucleic acid molecule of Claim 4, wherein said isolated nucleic acid molecule encodes a protective sequence product.

7. The isolated nucleic acid molecule of Claim 5, wherein said isolated nucleic acid molecule encodes a protective sequence product.

8. A vector comprising the nucleic acid of any one of Claims 1 and 2.

9. The vector of claim 8, wherein said vector is a viral vector.

10. An expression vector comprising the nucleic acid of any one of Claims 1 and 2 operatively associated with a regulatory nucleic acid controlling the expression of the nucleic acid in a host cell.

11. A host cell genetically engineered to contain the nucleic acid of any one of Claims 1 and 2.

12. A host cell genetically engineered to express the nucleic acid of any one of Claims 1 and 2 operatively associated with a regulatory nucleic acid controlling expression of the nucleic acid in said host cell.

13. The host cell of Claim 12, wherein said host cell is a neuronal cell.

14. The host cell of Claim 13, wherein said neuronal cell is a PC-12 cell or a primary dissociated neuron.

15. A transgenic, non-human animal which has been genetically engineered to contain a transgene comprising the nucleic acid of any one of Claims 1 and 2.

16. The transgenic, non-human animal of Claim 15, wherein the transgene is expressed.

17. An isolated polypeptide comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

18. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 4.

19. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 5.

20. An isolated fusion polypeptide comprising a fusion peptide and an amino acid sequence comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

21. An isolated fusion polypeptide comprising a fusion peptide and an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 4.

22. An isolated fusion polypeptide comprising a fusion peptide and an amino acid sequence encoded by the isolated nucleic acid molecules of Claim 5.

23. An antibody which binds to the isolated polypeptide of Claim 17.

24. A method for diagnosing a protective sequence-mediated condition, disorder or disease in an individual comprising obtaining a biological sample from said individual; contacting said biological sample with the antibody according to claim 23; wherein if said antibody interacts with said biological sample, but does not interact with a biological sample from a control individual not undergoing a protective sequence-mediated condition, disorder or disease, then a protective sequence-mediated condition, disorder or disease has been diagnosed.

25. A diagnostic kit for detecting a protective sequence-mediated condition, disorder or disease in an individual comprising a reagent in suitable packaging, wherein said reagent comprises the antibody according to claim 23.

26. A method for treating, ameliorating or preventing a protective sequence-mediated condition, disorder or disease in an individual comprising administering to the individual a compound which modulates the function, activity, expression and/or level of a protective sequence in a cell, cells, tissue, organ, organism or individual.

27. The method of Claim 26, wherein the compound inhibits the function, activity, expression and/or level of a protective sequence in a cell, cells, tissue, organ, organism or individual.

28. The method of Claim 26, wherein the compound enhances or potentiates the function, activity, expression and/or level of a protective sequence in a cell, cells, tissue, organ, organism or individual.

29. The methods of any one of Claims 26-28, wherein the compound is selected from the group consisting of a small organic molecule, an antibody, a ribozyme, an antisense molecule, and combinations thereof.

30. The method of any one of Claims 26-28, wherein the protective sequence-mediated condition, disorder, or disease is a condition, disorder, or disease of the central nervous system.

31. The method of Claim 30, wherein the central nervous system condition is an ischemia-related condition.

32. The method of Claim 31, wherein the central nervous system condition is a stroke.

33. The method of Claim 26, wherein the protective sequence encodes a polypeptide comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);
(b) an amino acid sequence shown in Figures 5(A-L);
(c) an amino acid sequence shown in Figures 6(A-K);
(d) an amino acid sequence shown in Figures 7(A-Z);
(e) an amino acid sequence shown in Figures 8(A-S);
(f) an amino acid sequence shown in Figures 9(A-X);
(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

34. The method of Claim 26, wherein the individual is a mammal.

35. The method of Claim 34, wherein the mammal is a human.

36. A method for treating, ameliorating, or preventing a protective sequence-mediated condition, disorder or disease in an individual comprising administering to the individual a compound which modulates the expression or activity of a protective sequence product and/or protective sequence regulatory product in the individual.

37. The method of Claim 36, wherein the compound inhibits the expression or activity of a protective sequence product and/or protective sequence regulatory product in the individual.

38. The method of Claim 36, wherein the compound enhances or potentiates the expression or activity of a protective sequence product and/or protective sequence regulatory product in the individual.

39. The method of Claim 36, wherein the compound is selected from the group consisting of a small organic molecule, an antibody, a ribozyme, an antisense molecule, and combinations thereof.

40. The method of Claim 36, wherein the protective sequence-mediated condition, disorder, or disease is a condition, disorder, or disease of the central nervous system.

41. The method of Claim 40, wherein the central nervous system condition is an ischemia-related condition.

42. The method of Claim 41, wherein the central nervous system condition is a stroke.

43. The method of Claim 36, wherein the protective sequence product comprises:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

44. The method of Claim 36, wherein the individual is a mammal.

45. The method of Claim 44, wherein the mammal is a human.

46. A method for identifying a compound which modulates expression of a protective sequence comprising:

(a) contacting a test compound to a cell that expresses a protective sequence;

(b) measuring a level of protective sequence expression in the cell;

(c) comparing the level of protective sequence expression in the cell in the presence of the test compound to a level of protective sequence expression in the cell in the absence of the test compound,

wherein if the level of protective sequence expression in the cell in the presence of the test compound differs from the level of expression of the protective sequence in the cell in the absence of the test compound, a compound that modulates expression of a protective sequence is identified.

47. The method of Claim 46, wherein the protective sequence is endogenously expressed within the cell.

48. The method of Claim 46, wherein the protective sequence encodes a polypeptide comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);
(b) an amino acid sequence shown in Figures 5(A-L);
(c) an amino acid sequence shown in Figures 6(A-K);
(d) an amino acid sequence shown in Figures 7(A-Z);
(e) an amino acid sequence shown in Figures 8(A-S);
(f) an amino acid sequence shown in Figures 9(A-X);
(g) an amino acid sequence shown in Figures 10(A-V);
(h) an amino acid sequence shown in Figures 11(A-I);
(i) an amino acid sequence shown in Figures 12(A-G); or
(j) the amino acid sequence shown in Figure 13(A-H).

49. The method of Claim 46, wherein the protective sequence comprises:

(a) a nucleic acid sequence shown in Figures 4(A-AB);
(b) a nucleic acid sequence shown in Figures 5(A-L);
(c) a nucleic acid sequence shown in Figures 6(A-K);
(d) a nucleic acid sequence shown in Figures 7(A-Z);
(e) a nucleic acid sequence shown in Figures 8(A-S);
(f) a nucleic acid sequence shown in Figures 9(A-X);
(g) a nucleic acid sequence shown in Figures 10(A-V);
(h) a nucleic acid sequence shown in Figures 11(A-I);
(i) a nucleic acid sequence shown in Figures 12(A-G);
(j) a nucleic acid sequence shown in Figure 13 (A-H); or
(k) a nucleic acid sequence shown in Figures 1(A-J).

50. A method for identifying a compound which modulates expression, function or activity of a protective sequence product or protective sequence regulatory element comprising:

(a) contacting a test compound to a cell that expresses a protective sequence product or protective sequence regulatory element;

(b) measuring a level of protective sequence product or protective sequence regulatory element expression, function or activity in the cell;

(c) comparing the level of protective sequence product or protective sequence regulatory element expression, function or activity in the cell in the presence of the test compound to a level of protective sequence product or protective sequence regulatory element expression or activity in the cell in the absence of the test compound,

wherein if the level of protective sequence product or protective sequence regulatory element expression, function or activity in the cell in the presence of the test compound differs from the level of protective sequence product or protective sequence regulatory element expression, function or activity in the cell in the absence of the test compound, a compound that modulates expression or activity of a protective sequence product or protective sequence regulatory element is identified.

51. The method of Claim 50, wherein the protective sequence product or protective sequence regulatory element comprises:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

52. A method for transferring a protective sequence into a cell comprising contacting the cell with a nucleic acid comprising a protective sequence such that the protective sequence is transferred into the cell.

53. The method of Claim 52 wherein the protective sequence is expressed in the cell.

54. The method of Claim 52 wherein the protective sequence delays and/or prevents the cell from undergoing cell death.

55. A method for modulating the function, activity, expression and/or level of a protective sequence in a cell comprising administering to the cell a compound which modulates the function, activity, expression and/or level of a protective sequence in the cell.

56. The method of Claim 55, wherein the compound inhibits the function, activity, expression and/or level of a protective sequence in the cell.

57. The method of Claim 55, wherein the compound enhances or potentiates the function, activity, expression and/or level of a protective sequence in the cell.

58. The methods of any one of Claims 55-57, wherein the compound is selected from the group consisting of a small organic molecule, an antibody, a ribozyme, an antisense molecule, and combinations thereof.

59. The method of Claim 55, wherein the protective sequence encodes a polypeptide comprising:

(a) an amino acid sequence shown in Figures 4(A-AB);

(b) an amino acid sequence shown in Figures 5(A-L);

(c) an amino acid sequence shown in Figures 6(A-K);

(d) an amino acid sequence shown in Figures 7(A-Z);

(e) an amino acid sequence shown in Figures 8(A-S);

(f) an amino acid sequence shown in Figures 9(A-X);

(g) an amino acid sequence shown in Figures 10(A-V);

(h) an amino acid sequence shown in Figures 11(A-I);

(i) an amino acid sequence shown in Figures 12(A-G); or

(j) the amino acid sequence shown in Figure 13(A-H).

60. A primer comprising an isolated nucleic acid molecule which hybridizes under highly stringent conditions to:

(a) a nucleic acid sequence shown in Figures 4(A-AB);

(b) a nucleic acid sequence shown in Figures 5(A-L);

(c) a nucleic acid sequence shown in Figures 6(A-K);

(d) a nucleic acid sequence shown in Figures 7(A-Z);

(e) a nucleic acid sequence shown in Figures 8(A-S);

(f) a nucleic acid sequence shown in Figures 9(A-X);

(g) a nucleic acid sequence shown in Figures 10(A-V);

(h) a nucleic acid sequence shown in Figures 11(A-I);

(i) a nucleic acid sequence shown in Figures 12(A-G);

(j) a nucleic acid sequence shown in Figure 13 (A-H); or

(k) a nucleic acid sequence shown in Figures 1(A-J).

61. A method for diagnosing a protective sequence-mediated condition, disorder or disease in an individual comprising obtaining a biological sample from said individual; contacting said biological sample with the primer according to claim 60; wherein if said primer interacts with said biological sample, but does not interact with a biological sample from a control individual not undergoing a protective sequence-mediated condition, disorder or disease, then a protective sequence-mediated condition, disorder or disease has been diagnosed.

62. A diagnostic kit for detecting a protective sequence-mediated condition, disorder or disease in an individual comprising a reagent in suitable packaging, wherein said reagent comprises the primer according to claim 60.

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tcgacccacg cgtccgcgga cgcgtgggta ggactttgaa gaatacagtt tcagtggagt   60
aaactatttt ttgtgatctg tttacttata ttatcctgac tttaaacatt ttttagcata  120
agaaaatagt aaaaaaatat tttaatgata taaaatcctt ggctgctagc taggagtcgc  180
tctgtgctat agtagaaaaa tatggagact gggagctgtg tgatctattt tcaccagtaa  240
ctgggtgact ttaaaaggcc tgtaacttgt acttgtctac ttttatccag ttctacactg  300
aaagattgtt tttgatgatt ctcaacatct ttttctggta tgtaagactt tcctcatgaa  360
attcagaaca ttgccattta aggaatggca aagatttttt ccctaaagtt aaaagatcaa  420
atatgaaatt aatataagtt ataaagtata tatttcttca acaataatgt acagttgaag  480
gtatgtcaaa aattgacttt catttataga aaaaaaagta aagtaggtaa ctgtattagt  540
tctctagggt agctgtaaca aaataccaaa aactgggtgg cttaaacagc aaaaaaatgt  600
attatctcac agttctgcag tctagaagtc tggaatcaag gtgttagtag ggctggttct  660
ttctgagggc tgcgaaggca ggatatgttc caggcctccc tctatggctt gtagatggcc  720
atcttcatgg tcacatggca ttctccctgt agctctctgt ttccagactt cccctttttg  780
taaggatatc agtgatatta gattagggtc ttccctaagg acctcatttg acctgcctgg  840
gctcaagcta ttctcccacc tctgcctccc taagagctgg gattacaggc atgagccatc  900
acacccgccc ctcattttaa tttgattacc tctgtaaata cctctgtctc caaatgagat  960
ttcatcctga gcagctgggg gttaggactt caatatatga atttgacagg gagggtagaa 1020
ggagagaaca gaattcaacc cacagcagca acaatctaat agcttcctgt gagcaagcaa 1080
agagaatgtt cattgtcagt ctcataggcg ccattcccta ttcatacgtt acttgtgctc 1140
tctcatattc cttgagtgtt ttaaattgta aacattcaag tacaaacaaa cttcgcttga 1200
ttaccagaga taaaaaagaa atgccttgta atttggtgtc atgtgaatgt tttaagtgga 1260
tacctgaaaa attgtactta agaatggcat aagagctttc tgattttcat tttacttcca 1320
ttaaagggga aaatatgcat agactgtcta tccattagcc agaacaatgg gacctctccc 1380
atcttaaaat aaaagccaaa ataatctggc caccaggaag aaagggtaga gcttgggaat 1440
gtcctcagga gattgtaaag atgcgtttcc ttgattcttt tgctcacact cttccctgtg 1500
actatttcct ccttcagggc tctatttctg ggttgggaga atgctgttcc agcaccaagc 1560
agtgtgggta tatatattca taccaaagag gcaatttgat tgtccttgga gttacaaaaa 1620
accaaatgtc aatgcctgat tagggaatac aacaataaaa gtaaaaataa tttaggagta 1680
tatatgcaga acatcagcct ttaaagtaat cttttattag gaaaatggca ttcacgattt 1740
gagaagatgg aaggtggtgg ggaacagaaa taaaaaaaaa aaaaaagggc ggcc       1794
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FIG.1A

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tcgacccacg cgtccgctac ccaggaaaca gctccatcag catcttagcc tgccccactc   60
tagccacaca tacccacgtg tactcctgag ttcagtgtgc ccacctcact cccacaccct  120
cacatagact tggcaagagt aaggagggaa ctccatagag acattttacc tatctcaggg  180
gagcagccac aaagaagcaa gtcttgtaaa aggtcttttg caaaggagag tgaacccagc  240
aatgagagat ccttaacagc tagtgcccat tagggggcta aacctaaagc ctgggtggtg  300
atggctcaaa cgctaatgag tcagtgaatc cttaccgacc ccctggcctt tataatctga  360
ggcaactttg gctgcagccc gggaatgtgc agggcactag ggaatacaag gccttcttcc  420
ctggttgtct tgtaataaaa cagccatggg gttgtccctc cagtccgaga gactgtgatg  480
aggcctacat agcagcgatg tggtcaggta aaaatcagga acccactgaa atcttgggca  540
agccaccctg cctgcttgtg cctcggttct ctcatatgtc atatatagga ggtgaggact  600
ccagctccac ctgccccagg tgggtgtggt gatgatgagg aaagacaaga ggcttgcaag  660
gaccctgaag aggtcggagc atcatacaga ttcctttatt agcccacatt ctgatgttcc  720
ctggtgagac ttgccccaag caattgctag taaatggggg ttaatttctt ctccacctcc  780
ctactgaaca aaaaaagaaa aagggcggcc                                   810
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FIG.1B

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taacagccca atatctacaa tcaaccaaca agtcattatt accctcactg tcaacccaac   60
acaggcatgc tcataaggaa aggttaaaaa aagtaaaagg aactcggcaa atcttacccc  120
gcctgtttac caaaaacatc acctctagca tcaccagtat tagaggcacc gcctgcccag  180
tgacacatgt ttaacggccg cggtacccta accgtgcaaa ggtagcataa tcacttgttc  240
cttaattagg gacctgtatg aatggctcca cgagggttca gctgtctctt acttttaacc  300
agtgaaattg acctgcccgt gaagaggcgg gcatgacaca gcaagacgag aagaccctat  360
ggagctttaa tttattaatg caaacagtac ctaacaaacc cacaggtcct aaactaccaa  420
acctgcatta aaaatttcgg ttggggcgac ctcggagcag aacccaacct ccgagcagta  480
catgctaaga cttcaccagt caaagcgaac tactatactc aattgatcca ataacttgac  540
caacggaaca agttacccta gggataacag cgcaatccta ttctagagtc catatcaaca  600
atagggttta cgacctcgat gttggatcag gacatcccga tggtgcagcc gctattaaag  660
gttcgtttgt tcaacgatta aagtcctacg tgatctgagt tcagaccgga gtaatccagg  720
tcggtttcta tctacttcaa attcctccct gtacgaaagg acaagagaaa taaggcctac  780
ttcacaaagc gccttccccc gtaaatgata tcatctcaac ttagtattat acccacaccc  840
acccaagaac agggtttaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  900
aaaaaaaaaa aagggcggcc                                              920
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FIG.1C

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tcgacccacg cgtccgcgca agatggcagc caccacgggc tcgggagtaa aagtccctcg   60
caatttccga ctgttggaag aactcgaaga aggccagaaa ggagtaggag atggcacagt  120
tagctggggt ctagaagatg acgaagacat gacacttaca agatggacag ggatgataat  180
tgggcctcca agaacaattt atgaaaaccg aatatacagc cttaaaatag aatgtggacc  240
taaataccca gaagcacccc cctttgtaag atttgtaaca aaaattaata tgaatggagt  300
aaatagttct aatggagtgg tggacccaag agccatatca gtgctagcaa aatggcagaa  360
ttcatatagc atcaaagttg tcctgcaaga gcttcggcgc ctaatgatgt ctaaagaaaa  420
tatgaaactc cctcagccgc ccgaaggaca gtgttacagc aattaatcaa aaagaaaaac  480
cacaggccct tccccttccc cccaattcga tttaatcagt cttcattttc cacagtagta  540
aattttctag atacgtcttg tagacctcaa agtaccggaa aggaagctcc cattcaaagg  600
aaatttatct taagatactg taaatgatac taatttttc gtccatttga aatatataag  660
ttgtgctata acaaatcatc ctgtcaagtg taaccactgt ccacgtagtt gaacttctgg  720
gatcaagaaa gtctatttaa attgattccc atcataactg gtggggcaca tctaactcaa  780
ctgtgaaaag acacatcaca caatcacctt gctgctgatt acacggcctg gggtctctgc  840
cttctcccct taccctcccg cctcccaccc tccctgcaac aacagccctc tagcctgggg  900
ggcttgttag agtagatgtg aaggtttcag gtcgcagcct gtgggactac tgctaggtgt  960
gtggggtgtt tcgcctgcac ccctggtttc tttaagtctt aagtgatgcc ccttccaaac 1020
catcatcctg tccccacgct cctccactcc cgcccttggc cgaagcatag attgtaaccc 1080
ctccactccc ctctgagatt ggccttcggt gaggaattca gggctttccc catatcttct 1140
ctcccccacc tttatcgagg ggtgctgctt tttctccctc ctcctcaagt tcctttttgc 1200
accgtcacca cccaacacct tccatgacac ttccttgctt tggccagaag ccatcaggta 1260
aggttggaaa gagcctctga cctcccttgt ttagttttgg aaccatactc actcactctc 1320
caccagcctg ggaaatgaat attgggtcct cagccctgcc accctctgct gtcatcagct 1380
gatgcattgt ttttagctca ggttttgata aggtgaaaag aatagtcacc agggttactc 1440
agacctgcca gctctcggag tccttggtgg ttgaacttgg agaaagaccg catgaagata 1500
cttgtaagca cacatgatcc ctctgaattg ttttactttc ctgtaactgc ttttgctttt 1560
aaaaattgaa gaagttttaa acagggcttt catttggtca tccttgcaat ccattggggt 1620
ctagtttgga atctgacaac tggaacaaaa agaaccttga atccggtgca tgccttggtt 1680
ttggtgctgc tgctgcttcc caagatcctc agcagggatt aagaaggaac ccggtgtgca 1740
cagcagatcc ccgaaattgg tgggcttgac ctcctggcaa attgctgcgt ctttccactt 1800
gctgttcagg accactaaat gctgaaatgt ggatgcatac cgaaataaaa gcaattcatt 1860
gtgtactaaa ggtttttttt tttttttaa tttagtattt gtgtaaaacc accttttgaa 1920
gcagcaacta tcaagtctga aaagcaattg atgtttccat taatcttttt ctggggggaa 1980
aaccttagtt ctaaggattt aacatcctgt aagtgaagtt taacataaca gtattccata 2040
agcagccttt ttattgtcag accattgcct gattttaata taataaaaaa aaagtgtgcg 2100
ttaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaagggc ggcc                  2144
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FIG.1D

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tcgacccacg cgtccggagg caggaaaatc gcttgaaccc aggaggtgga ggttgtagtg   60
agccgagatc acaccactgc actccagcct gggtgacaag aacgaaactc catctcaaaa  120
aataaaataa aatatatact atcttgctcc tcagaaccag tggggaagaa gagggaaggc  180
aaagaaagaa actgagcata gtaaacacag catttttttg taggctctta tttaaaatgt  240
gtgtgtgtgt gtgtatgtgt gtgtttctga gtaagtattg actgggaaaa agagagaagt  300
caatcaaaag tatactgtgc aattgagaga ggctggccca agatttaaaa cttcctgtgg  360
gtaatctaac tgtgagtaga taggaatcgg ccatatgacg aaatgagatc aataggaaat  420
gtgctttttg aggaaatttt attttagtac caaatgttgc cagtgacaat cttcagttaa  480
gaagtaagtt atttgaccta aaattcttat ctctgccact ttggtttaaa aacaaaaacc  540
cttatataca tggaatagtt atattttaat taagcattta ttttagttgt tttcatccat  600
tcaagcaaaa tgaataagca gcatttttca ttgcacttaa aaatgtaaaa tacctgcatg  660
ccactaatct gtaacatttt accagttcag atgcctgtaa tgtgtgactt tatgtgtgtc  720
tgtgttgttt tgaagagaat aaaggaaata atactttgca aactgtttaa acaagtgttt  780
aaacttctat tggcaacatt tattgggcta agcagttatt gaaaactccg catagtttta  840
ttttccattt gaaacttcaa tcaaatcaag actattatat tcattaggga attaaagact  900
aatttgcttt ttaaatgtga agtggaacac tgtgtggaaa gtaaatgtgt gatgaagcaa  960
aatgtataaa gtatgaaata ttatactttt accctggata attattcagg accccagttg 1020
gcccaaatag gtgcaatttt taatcctttg aaattagcca gccagaccta atgctaaggt 1080
aaatgtaaac tgttttaatt aattaagatc tttctgcttt cgaaggtata atgtatctat 1140
ttctgtcagg aatgatattt ccaaatgaaa atgtaaagaa cattgggaaa taataaactt 1200
tcctttcaaa gtaaaagtaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1260
aaaaaaaaaa aaaaaaaaaa aaaaagggcg gcc                              1293
```

FIG.1E

```
tcgacccacg cgtccggttg ttaaacccta tgaagagtaa cagtgtagac cagactgcct   60
ctctcagata tgtgcctgat attttgtgga tacctcccct gcactggcaa aacactatgc  120
ttttgggtgt tagactgaaa tattttaaga gtatttaacc tttccagtat tctgtttcac  180
gcttagatgg aaatgtatct tatgaataga gacatattaa aataatgttt acatcttaga  240
aaaaacatag atagtgctag taatattact tataactgta atatatagat tcagaaatac  300
attttcatta tccaaaatca gcttcaacaa atggtttctg gagacaaata atttgttttc  360
attatcattg tataatcagg ttaatgattt attttttgac taaatgtgca atttcttatc  420
actagataac tttcagtatc agtggtggtt acttattact taaatcagag gaaggatttt  480
ataaagatta ataaatttaa ttttaccaat aaatattccc ataatttaga aaaggatgtc  540
gacttgctaa tttcagaaat aattattcat ttttaaaaag cccctttttaa agcatctact  600
tgaagattgg tataattttc ataaaatgtc ttttttttta gtgtcccaaa gatatcttag  660
ataaactatt ttgaagttca gatttcagat gaggcaacat tttcttgaga taattaccca  720
agtttcatcc atgttgaatg gtacaaaata tttctgtgaa actaacagga agatattttc  780
agataactag gataacttgt tgctttgtta cccagcctaa ttgaagagtg gcagagacta  840
ctacaaaaag caacctttttc attttcacta agagtttaaa agctattgta ttattaaaaa  900
gtctttacaa tgcttgtttc aaagaaccaa cagaaaaaaa agctaagaaa actgagaact  960
aacattaaaa aaattaaatt tagaataaga atgatttctt taatttgtcc ttttttttctt 1020
tggtctaaaa cattattaaa tttttgtaaa tattttgatt taatgtgtct tagatcctca 1080
ttattttaat acaggaaaag aaaagattta gtaatttctt accatgctaa tatgtaaagt 1140
tcatgccatc caggcattta agagcgatcc tcatcccttc agcaatatgt atttgagttc 1200
acactatttc tgttttacag cagttttgaa aaacacatac tatgccacca attgtcatat 1260
tatttttaga tgatgtaaca tagccatcaa aattaatatt atgtaatgcc taatacttag 1320
tatgtaaatg tcacgagatc atttttacat taaacgtgaa aaaaaatcaa aaaaaaaaaa 1380
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1440
aaaaaaaaaa aaaaaaaagg gcggcc                                       1466
```

FIG. 1F

```
tcgacccacg cgtccgccca cgcgtccgca agagcatctt cagcgggcga gtccccggct   60
cctccagctc cttcctcctc ttcctcctcc tcctccacct ccggcttttg ggggatcact  120
gtcctctctc ggcagcagaa tgagccggca ggtggtccgc tccagcaagt tccgccacgt  180
gtttggacag ccggccaagg ccgaccagtg ctatgaagat gtgcgcgtct cacagaccac  240
ctgggacagt ggcttctgtg ctgtcaaccc taagtttgtg gccctgatct gtgaggccag  300
cgggggaggg gccttcctgg tgctgcccct gggcaagact ggacgtgtgg acaagaatgc  360
gcccacggtc tgtggccaca cagcccctgt gctagacatc gcctggtgcc cgcacaatga  420
caacgtcatt gccagtggct ccgaggactg cacagtcatg gtgtgggaga tcccagatgg  480
gggcctgatg ctgcccctgc gggagcccgt cgtcaccctg gagggccaca ccaagcgtgt  540
gggcattgtg gcctggcaca ccacagccca gaacgtgctg ctcagtgcag gttgtgacaa  600
cgtgatcatg gtgtgggacg tgggcactgg ggcggccatg ctgacactgg gcccagaggt  660
gcacccagac acgatctaca gtgtggactg gagccgagat ggaggcctca tttgtacctc  720
ctgccgtgac aagcgcgtgc gcatcatcga gccccgcaaa ggcactgtcg tagctgagaa  780
ggaccgtccc cacgagggga cccggcccgt gcgtgcagtg ttcgtgtcgg aggggaagat  840
cctgaccacg ggcttcagcc gcatgagtga gcggcaggtg gcgctgtggg acacaaagca  900
cctggaggag ccgctgtccc tgcaggagct ggacaccagc agcggtgtcc tgctgccctt  960
ctttgaccct gacaccaaca tcgtctacct ctgtggcaag ggtgacagct caatccggta 1020
ctttgagatc acttccgagg cccctttcct gcactatctc tccatgttca gttccaagga 1080
gtcccagcgg ggcatgggct acatgcccaa acgtggcctg gaggtgaaca agtgtgagat 1140
cgccaggttc tacaagctgc acgagcggag gtgtgagccc attgccatga cagtgcctcg 1200
aaagtcggac ctgttccagg aggacctgta cccacccacc gcagggcccg accctgccct 1260
cacggctgag gagtggctgg ggggtcggga tgctgggccc ctcctcatct ccctcaagga 1320
tggctacgta cccccaaaga gccgggagct gagggtcaac cggggcctgg acaccgggcg 1380
caggagggca gcaccagagg ccagtggcac tcccagctcg gatgccgtgt ctcggctgga 1440
ggaggagatg cggaagctcc aggccacggt gcaggagctc cagaagcgct tggacaggct 1500
ggaggagaca gtccaggcca agtagagccc cgcagggcct ccagcagggt cagccattca 1560
cacccatcca ctcacctccc attcccagcc acatggcaga gaaaaaaatc ataataaaat 1620
ggctttattt tctggtaaaa aaaaaaaaaa agggcggcc                        1659
```

FIG.1G

```
tcgacccacg cgtccgctct gccgcagcct gtgccgccgc cgcctcctgg gaagagagga   60
agcgggagag gagcccacgt ctcctgtcac ccaatatctc cagccgcgca gtcccgaaga  120
gtgtaagatg ttcgcctgcg ccaagctcgc ctgcaccccc tctctgatcc gagctggatc  180
cagagttgca tacagaccaa tttctgcatc agtgttatct cgaccagagg ctagtaggac  240
tggagagggc tctacggtat ttaatggggc ccagaatggt gtgtctcagc taatccaaag  300
ggagtttcag accagtgcaa tcagcagaga cattgatact gctgccaaat ttattggtgc  360
aggtgctgca acagtaggag tggctggttc tggtgctggt attggaacag tctttggcag  420
ccttatcatt ggttatgcca gaaacccttc gctgaagcag cagctgttct catatgctat  480
cctgggattt gccttgtctg aagctatggg tctcttttgt ttgatggttg ctttcttgat  540
tttgtttgcc atgtaacaaa ttactgcttg acatgttggc attcatatta attacggatg  600
taattctgtg tatcttactg tgactccgaa aactgtagta ttggtgtcat gggaatgtac  660
gttatttcca aagtcatttc attaaagatg aaaactttaa aaaaaaaaaa aaaagggcgg  720
cc                                                                  722
```

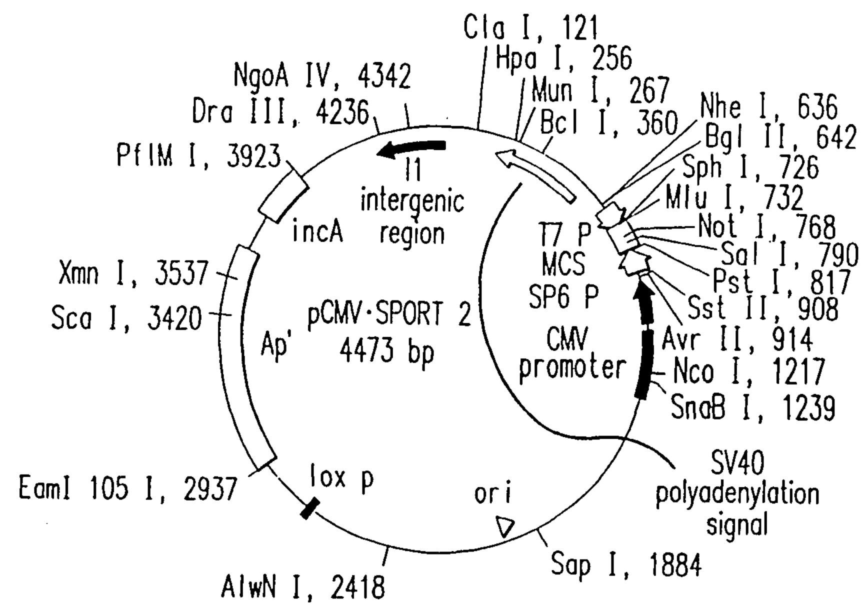
FIG. 1H

```
tcgacccacg cgtccggatt tagcaggaag ctgtgagagc agtttggttt ctagcatgaa   60
gacagagccc caccctcaga tgcacatgag ctggcgggat tgaaggatgc tgtcttcgta  120
ctgggaaagg gattttcagc cctcagaatc gctccacctt gcagctctcc ccttctctgt  180
attcctagaa actgacacat gctgaacatc acagcttatt tcctcatttt tataatgtcc  240
cttcacaaac ccagtgtttt aggagcatga gtgccgtgtg tgtgcgtcct gtcggagccc  300
tgtctcctct ctctgtaata aactcatttc tagcagaaaa aaaaaaaaaa aaaaaagggc  360
ggcc                                                                364
```

FIG.1I

```
tccgctctta gtaagattac acatgcaagc atccccgttc cagtgagttc accctctaaa   60
tcaccacgat caaaagggac aagcatcaag cacgcagcaa tgcagctcaa aacgcttagc  120
ctagccacac ccccacggga aacagcagtg attaaccttt agcaataaac gaaagtttaa  180
ctaagctata ctaaccccag ggttggtcaa tttcgtgcca gccaccgcgg tcacacgatt  240
aacccaagtc aatagaagcc ggcgtaaaga gtgttttaga tcacccccte cccaataaag  300
ctaaaactca cctgagttgt aaaaaactcc agttgacaca aaatagacta cgaaagtggc  360
tttaacatat ctgaacacac aatagctaag acccaaactg ggattagata ccccactatg  420
cttagcccta aacctcaaca gttaaatcaa caaaactgct cgccagaaca ctacgagcca  480
cagcttaaaa ctcaaaggac ctggcggtgc ttcatacccc tctagaggag cctgttctgt  540
aatcgataaa ccccgatcaa cctcaccacc tcttgctcag cctatatacc gccatcttca  600
gcaaaccctg atgaaggcta caaagtaagc gcaagtaccc acgtaaagac gttaggtcaa  660
ggtgtagccc atggggtggc aagaaatggg ctacattttc taccccagaa aactacgata  720
gcccttatga aacttaaggg tcgaaggtgg atttagcagt aaactgagag tagagtgctt  780
agttgaacag ggccctgaag cgcgtacaca ccgcccgtca ccctcctcaa gtatacttca  840
aaggacattt aactaaaacc cctacgcatt tatatagagg agacaagtcg taacatggta  900
agtgtactgg aaagtgcact tggacgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
aaaaaaaaaa aaaaaaaagg gcggcc                                     1046
```

FIG.1J




Multiple Cloning Site (MCS) of Plasmid pCMV*SPORT 2 after Cloning of a cDNA Insert

636 Forward sequencing primer T7 promoter

CTAGCAGATC TCCCAGTCAC GACGTTGTAA AACGACGGCC AGTGCCTAGC TTATAATACG ACTCACTATA GGGAGAGAGC

Sph I Mlu I Hind III Apa I Xho I BamH I Xba I Not I Mlu 1*

TATGACGTCG CATGCACGCG TAAGCTTGGG CCCCTCGAGG GATCCTCTAG AGCGGCCGC cDNA Insert CGGACGCGTG

Sal I Smi I EcoR I Rsr II Kpn I Pst I SP6 promoter

GGTCGACCCG GGAATTCCGG ACCGGTACCT GCAGGCGTAC CTTCTATAGT GTCACCTAAA TAGCTTTTTG CAAAAGCCTA

Reverse sequencing primer

GTGGTCATAG CTGTTTCCTG TGTGAAATTG TTATCCGCTC CGCGGCCTAG GCTAGAGTCC CMV PROMOTER

*This Mlu I restriction site contained within the Sal I adapter is introduced into the pCMV*SPORT 2 vector upon ligation of the cDNA insert. Due to flanking sites, Mlu I, by itself, or the combined Not I–Sal I digestion can be used to completely excise the cDNA insert.

FIG.2

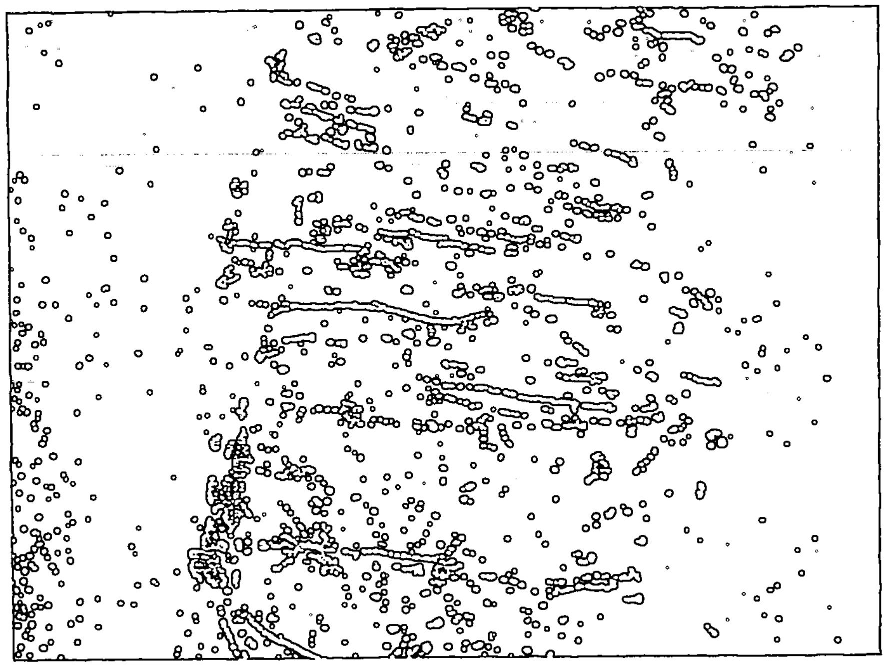


FIG.3A

FIG.3B

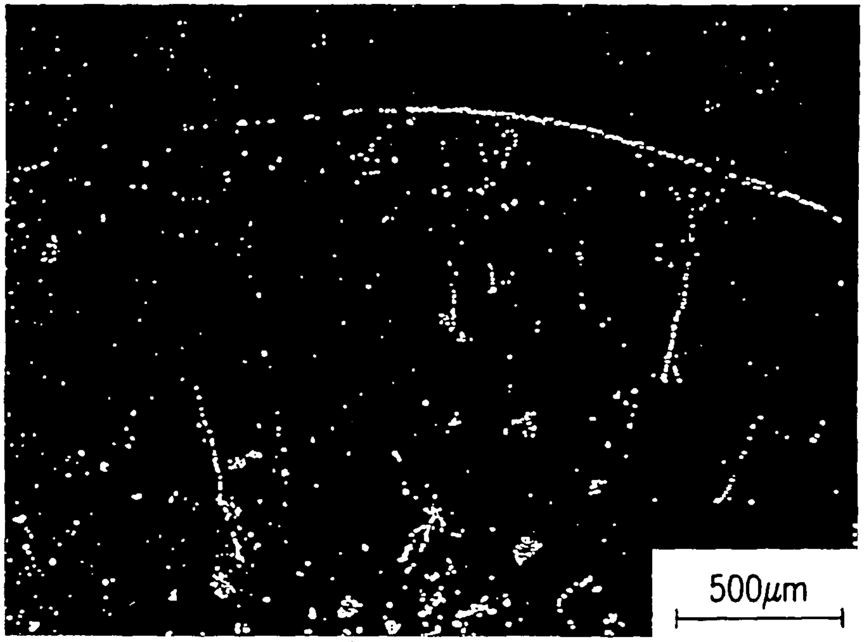



FIG.3C

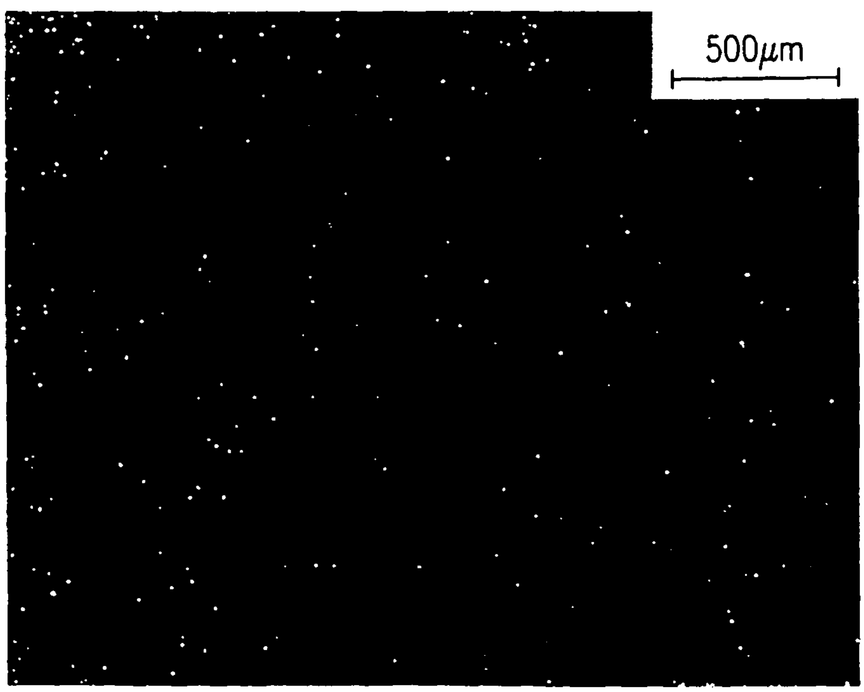



FIG.3D

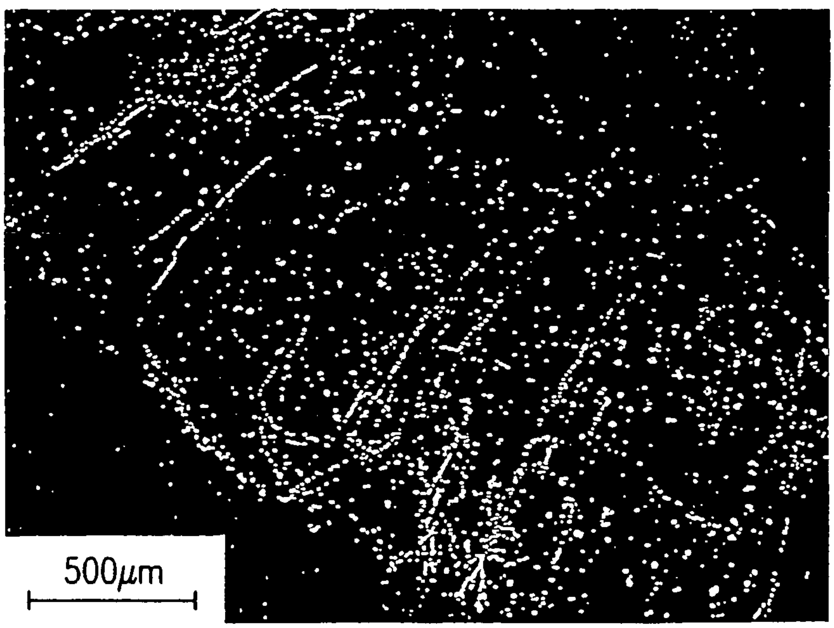



FIG.3E

COMPARISON OF NEURONAL PROTECTION IN STROKED RAT CORTICAL BRAIN SLICES

| SEQUENCE DESIGNATION | AVERAGE SURVIVING NEURONS PER SLICE (n) @ 3 DAYS POST-STROKE |
|---|---|
| EGFP CONTROL (NEGATIVE CONTROL) | 1.01 (41) |
| CNI-00718 | 20.6 (6) |
| CNI-00722 | 21 (6) |
| CNI-00725 | 14.3 (9) |
| CNI-00726 | 19.7 (3) |
| CNI-00727 | 17.8 (9) |
| CNI-00728 | 10.7 (3) |
| CNI-00729 | 13 (3) |
| CNI-00730 | 14.7 (3) |
| CNI-00731 | 11.7 (3) |
| CNI-00732 | 12.7 (3) |

FIG. 3F

atggagactg ggagctgtgt gatctatttt caccagtaa 39

Met Glu Thr Gly Ser Cys Val Ile Tyr Phe His Gln
1 5 10

FIG.4A

atgattctca acatcttttt ctggtatgta agactttcct catga 45

Met Ile Leu Asn Ile Phe Phe Trp Tyr Val Arg Leu Ser Ser
1 5 10

FIG.4B

atgaaattca gaacattgcc atttaaggaa tggcaaagat tttttcccta a 51

Met Lys Phe Arg Thr Leu Pro Phe Lys Glu Trp Gln Arg Phe Phe Pro
1 5 10 15

FIG.4C

atggcaaaga tttttttccct aaagttaaaa gatcaaatat ga 42

Met Ala Lys Ile Phe Ser Leu Lys Leu Lys Asp Gln Ile
1 5 10

FIG.4D

atgaaattaa tataa 15

Met Lys Leu Ile
1

FIG.4E

atgtacagtt ga 12

Met Tyr Ser
1

FIG.4F

```
atgtcaaaaa ttgactttca tttatag   27

Met Ser Lys Ile Asp Phe His Leu
 1               5
```

FIG.4G

```
atgtattatc tcacagttct gcagtctaga agtctggaat caaggtgtta g   51

Met Tyr Tyr Leu Thr Val Leu Gln Ser Arg Ser Leu Glu Ser Arg Cys
 1               5                  10                  15
```

FIG.4H

```
atgttccagg cctccctcta tggcttgtag   30

Met Phe Gln Ala Ser Leu Tyr Gly Leu
 1               5
```

FIG.4I

```
atggcttgta gatggccatc ttcatggtca catggcattc tccctgtagc tctctgtttc    60
cagacttccc ctttttgtaa ggatatcagt gatattagat tagggtcttc cctaaggacc   120
catttgacct gcctgggctc aagctattct cccacctctg cctccctaag agctgggatt   180
acaggcatga gccatcacac ccgcccctca ttttaatttg a                       221

Met Ala Cys Arg Trp Pro Ser Ser Trp Ser His Gly Ile Leu Pro Val
 1               5                  10                  15
Ala Leu Cys Phe Gln Thr Ser Pro Phe Cys Lys Asp Ile Ser Asp Ile
            20                  25                  30
Arg Leu Gly Ser Ser Leu Arg Thr Ser Phe Asp Leu Pro Gly Leu Lys
        35                  40                  45
Leu Phe Ser His Leu Cys Leu Pro Lys Ser Trp Asp Tyr Arg His Glu
    50                  55                  60
Pro Ser His Pro Pro Leu Ile Leu Ile
65                  70
```

FIG.4J

atggccatct tcatggtcac atggcattct ccctgtagct ctctgtttcc agacttcccc 60
tttttgtaa 69

Met Ala Ile Phe Met Val Thr Trp His Ser Pro Cys Ser Ser Leu Phe
1 5 10 15
Pro Asp Phe Pro Phe Leu
20

FIG.4K

atggtcacat ggcattctcc ctgtagctct ctgtttccag acttcccctt tttgtaa 57
Met Val Thr Trp His Ser Pro Cys Ser Ser Leu Phe Pro Asp Phe Pro
1 5 10 15
Phe Leu

FIG.4L

atggcattct ccctgtag 18

Met Ala Phe Ser Leu
1 5

FIG.4M

atgagccatc acacccgccc ctcattttaa 30

Met Ser His His Thr Arg Pro Ser Phe
1 5

FIG.4N

```
atgagatttc atcctgagca gctgggggtt aggacttcaa tatatgaatt tgacagggag   60
ggtagaagga gagaacagaa ttcaacccac agcagcaaca atctaatagc ttcctgtgag  120
caagcaaaga gaatgttcat tgtcagtctc ataggcgcca ttccctattc atacgttact  180
tgtgctctct catattcctt gagtgtttta aattgtaaac attcaagtac aaacaaactt  240
cgcttgatta ccagagataa aaaagaaatg ccttgtaatt tggtgtcatg tgaatgtttt  300
aagtggatac ctgaaaaatt gtacttaaga atggcataa                         339
```

```
Met Arg Phe His Pro Glu Gln Leu Gly Val Arg Thr Ser Ile Tyr Glu
 1               5                  10                  15
Phe Asp Arg Glu Gly Arg Arg Arg Glu Gln Asn Ser Thr His Ser Ser
            20                  25                  30
Asn Asn Leu Ile Ala Ser Cys Glu Gln Ala Lys Arg Met Phe Ile Val
        35                  40                  45
Ser Leu Ile Gly Ala Ile Pro Tyr Ser Tyr Val Thr Cys Ala Leu Ser
    50                  55                  60
Tyr Ser Leu Ser Val Leu Asn Cys Lys His Ser Ser Thr Asn Lys Leu
65                  70                  75                  80
Arg Leu Ile Thr Arg Asp Lys Lys Glu Met Pro Cys Asn Leu Val Ser
                85                  90                  95
Cys Glu Cys Phe Lys Trp Ile Pro Glu Lys Leu Tyr Leu Arg Met Ala
            100                 105                 110
```

FIG.4O

```
atgaatttga cagggagggt agaaggagag aacagaattc aacccacagc agcaacaatc   60
taa                                                                  63
```

```
Met Asn Leu Thr Gly Arg Val Glu Gly Glu Asn Arg Ile Gln Pro Thr
 1               5                  10                  15
Ala Ala Thr Ile
            20
```

FIG.4P

```
atgttcattg tcagtctcat aggcgccatt ccctattcat acgttacttg tgctctctca   60
tattccttga gtgttttaaa ttgtaaacat tcaagtacaa acaaacttcg cttgattacc  120
agagataaaa aagaaatgcc ttgtaatttg gtgtcatgtg aatgttttaa gtggatacct  180
gaaaaattgt acttaagaat ggcataa                                      207

Met Phe Ile Val Ser Leu Ile Gly Ala Ile Pro Tyr Ser Tyr Val Thr
 1               5                  10                  15
Cys Ala Leu Ser Tyr Ser Leu Ser Val Leu Asn Cys Lys His Ser Ser
            20                  25                  30
Thr Asn Lys Leu Arg Leu Ile Thr Arg Asp Lys Lys Glu Met Pro Cys
        35                  40                  45
Asn Leu Val Ser Cys Glu Cys Phe Lys Trp Ile Pro Glu Lys Leu Tyr
    50                  55                  60
Leu Arg Met Ala
65
```

FIG.4Q

```
atgccttgta atttggtgtc atgtgaatgt tttaagtgga tacctgaaaa attgtactta   60
agaatggcat aa                                                        72

Met Pro Cys Asn Leu Val Ser Cys Glu Cys Phe Lys Trp Ile Pro Glu
 1               5                  10                  15
Lys Leu Tyr Leu Arg Met Ala
            20
```

FIG.4R

```
atgcatagac tgtctatcca ttag   24

Met His Arg Leu Ser Ile His
 1               5
```

FIG.4S

```
atgggacctc tcccatctta a   21

Met Gly Pro Leu Pro Ser
 1               5
```

FIG.4T

atgtcctcag gagattgtaa agatgcgttt ccttga 36

Met Ser Ser Gly Asp Cys Lys Asp Ala Phe Pro
1 5 10

FIG.4U

atgcgtttcc ttgattcttt tgctcacact cttccctgtg actatttcct ccttcagggc 60
tctatttctg ggttgggaga atgctgttcc agcaccaagc agtgtgggta tatatattca 120
taccaaagag gcaatttgat tgtccttgga gttacaaaaa accaaatgtc aatgcctgat 180
tag 183

Met Arg Phe Leu Asp Ser Phe Ala His Thr Leu Pro Cys Asp Tyr Phe
1 5 10 15
Leu Leu Gln Gly Ser Ile Ser Gly Leu Gly Glu Cys Cys Ser Ser Thr
20 25 30
Lys Gln Cys Gly Tyr Ile Tyr Ser Tyr Gln Arg Gly Asn Leu Ile Val
35 40 45
Leu Gly Val Thr Lys Asn Gln Met Ser Met Pro Asp
50 55 60

FIG.4V

atgctgttcc agcaccaagc agtgtgggta tatatattca taccaaagag gcaatttgat 60
tgtccttgga gttacaaaaa accaaatgtc aatgcctga 99

Met Leu Phe Gln His Gln Ala Val Trp Val Tyr Ile Phe Ile Pro Lys
1 5 10 15
Arg Gln Phe Asp Cys Pro Trp Ser Tyr Lys Lys Pro Asn Val Asn Ala
20 25 30

FIG.4W

atgtcaatgc ctgattag 18

Met Ser Met Pro Asp
1 5

FIG.4X

```
atgcctgatt ag    12

Met Pro Asp
 1
```

FIG.4Y

```
atgcagaaca tcagccttta a    21

Met Gln Asn Ile Ser Leu
 1                  5
```

FIG.4Z

```
atggcattca cgatttga    18

Met Ala Phe Thr Ile
 1              5
```

FIG.4AA

```
atggaaggtg gtggggaaca gaaataa    27

Met Glu Gly Gly Gly Glu Gln Lys
 1              5
```

FIG.4AB

atgagagatc cttaa 15

Met Arg Asp Pro
1

FIG.5A

atggctcaaa cgctaatgag tcagtga 27

Met Ala Gln Thr Leu Met Ser Gln
1 5

FIG.5B

atgagtcagt ga 12

Met Ser Gln
1

FIG.5C

atgtgcaggg cactagggaa tacaaggcct tcttccctgg ttgtcttgta a 51

Met Cys Arg Ala Leu Gly Asn Thr Arg Pro Ser Ser Leu Val Val Leu
1 5 10 15

FIG.5D

atggggttgt ccctccagtc cgagagactg tga 33

Met Gly Leu Ser Leu Gln Ser Glu Arg Leu
1 5 10

FIG.5E

atgaggccta catag 15

Met Arg Pro Thr
1

FIG.5F

atgtggtcag gtaaaaatca ggaacccact gaaatcttgg gcaagccacc ctgcctgctt 60
gtgcctcggt tctctcatat gtcatatata ggaggtgagg actccagctc cacctgcccc 120
aggtgggtgt ggtga 135

Met Trp Ser Gly Lys Asn Gln Glu Pro Thr Glu Ile Leu Gly Lys Pro
1 5 10 15
Pro Cys Leu Leu Val Pro Arg Phe Ser His Met Ser Tyr Ile Gly Gly
20 25 30
Glu Asp Ser Ser Ser Thr Cys Pro Arg Trp Val Trp
35 40

# FIG.5G

atgtcatata taggaggtga ggactccagc tccacctgcc ccaggtgggt gtggtga 57

Met Ser Tyr Ile Gly Gly Glu Asp Ser Ser Ser Thr Cys Pro Arg Trp
1 5 10 15
Val Trp

# FIG.5H

atgatgagga aagacaagag gcttgcaagg accctgaaga ggtcggagca tcatacagat 60
tcctttatta gcccacattc tgatgttccc tggtga 96

Met Met Arg Lys Asp Lys Arg Leu Ala Arg Thr Leu Lys Arg Ser Glu
1 5 10 15
His His Thr Asp Ser Phe Ile Ser Pro His Ser Asp Val Pro Trp
20 25 30

# FIG.5I

atgaggaaag acaagaggct tgcaaggacc ctgaagaggt cggagcatca tacagattcc 60
tttattagcc cacattctga tgttccctgg tga 93

Met Arg Lys Asp Lys Arg Leu Ala Arg Thr Leu Lys Arg Ser Glu His
1 5 10 15
His Thr Asp Ser Phe Ile Ser Pro His Ser Asp Val Pro Trp
20 25 30

# FIG.5J

atgttccctg gtgagacttg ccccaagcaa ttgctagtaa atgggggtta a 51

Met Phe Pro Gly Glu Thr Cys Pro Lys Gln Leu Leu Val Asn Gly Gly
1 5 10 15

FIG.5K

atgggggtta atttcttctc cacctcccta ctgaacaaaa aaagaaaaag ggcggcc 57

Met Gly Val Asn Phe Phe Ser Thr Ser Leu Leu Asn Lys Lys Arg Lys
1 5 10 15
Arg Ala Ala

FIG.5L

atgctcataa ggaaaggtta a 21

Met Leu Ile Arg Lys Gly
1 5

FIG.6A

atgtttaacg gccgcggtac cctaaccgtg caaaggtag 39

Met Phe Asn Gly Arg Gly Thr Leu Thr Val Gln Arg
1 5 10

FIG.6B

atgaatggct ccacgagggt tcagctgtct cttactttta accagtga 48

Met Asn Gly Ser Thr Arg Val Gln Leu Ser Leu Thr Phe Asn Gln
1 5 10 15

FIG.6C

atggctccac gagggttcag ctgtctctta cttttaacca gtgaaattga cctgcccgtg 60
aagaggcggg catga 75

Met Ala Pro Arg Gly Phe Ser Cys Leu Leu Leu Leu Thr Ser Glu Ile
1 5 10 15
Asp Leu Pro Val Lys Arg Arg Ala
20

FIG.6D

atgacacagc aagacgagaa gaccctatgg agctttaatt tattaatgca aacagtacct 60
aacaaaccca caggtcctaa actaccaaac ctgcattaa 99

Met Thr Gln Gln Asp Glu Lys Thr Leu Trp Ser Phe Asn Leu Leu Met
1 5 10 15
Gln Thr Val Pro Asn Lys Pro Thr Gly Pro Lys Leu Pro Asn Leu His
20 25 30

FIG.6E

atggagcttt aa 12

Met Glu Leu
1

FIG.6F

```
atgcaaacag tacctaacaa acccacaggt cctaaactac caaacctgca ttaa    54

Met Gln Thr Val Pro Asn Lys Pro Thr Gly Pro Lys Leu Pro Asn Leu
 1              5                  10                  15
His
```

FIG.6G

```
atgctaagac ttcaccagtc aaagcgaact actatactca attga    45

Met Leu Arg Leu His Gln Ser Lys Arg Thr Thr Ile Leu Asn
 1              5                  10
```

FIG.6H

```
atgttggatc aggacatccc gatggtgcag ccgctattaa aggttcgttt gttcaacgat    60
taa                                                                   63

Met Leu Asp Gln Asp Ile Pro Met Val Gln Pro Leu Leu Lys Val Arg
 1              5                  10                  15
Leu Phe Asn Asp
            20
```

FIG.6I

```
atggtgcagc cgctattaaa ggttcgtttg ttcaacgatt aa    42

Met Val Gln Pro Leu Leu Lys Val Arg Leu Phe Asn Asp
 1              5                  10
```

FIG.6J

```
atgatatcat ctcaacttag tattataccc acacccaccc aagaacaggg tttaaaaaaa    60
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagg gcggcc       116

Met Ile Ser Ser Gln Leu Ser Ile Ile Pro Thr Pro Thr Gln Glu Gln
 1              5                  10                  15
Gly Leu Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
            20                  25                  30
Lys Lys Lys Lys Gly Arg
        35
```

FIG.6K

```
atggcagcca ccacgggctc gggagtaaaa gtccctcgca atttccgact gttggaagaa   60
ctcgaagaag gccagaaagg agtaggagat ggcacagtta gctggggtct agaagatgac  120
gaagacatga cacttacaag atggacaggg atgataattg ggcctccaag aacaatttat  180
gaaaaccgaa tatacagcct taaaatagaa tgtggaccta aatacccaga agcacccccc  240
tttgtaagat ttgtaacaaa aattaatatg aatggagtaa atagttctaa tggagtggtg  300
gacccaagag ccatatcagt gctagcaaaa tggcagaatt catatagcat caaagttgtc  360
ctgcaagagc ttcggcgcct aatgatgtct aaagaaaata tgaaactccc tcagccgccc  420
gaaggacagt gttacagcaa ttaa                                         444
```

```
Met Ala Ala Thr Thr Gly Ser Gly Val Lys Val Pro Arg Asn Phe Arg
 1               5                  10                  15
Leu Leu Glu Glu Leu Glu Glu Gly Gln Lys Gly Val Gly Asp Gly Thr
            20                  25                  30
Val Ser Trp Gly Leu Glu Asp Asp Glu Asp Met Thr Leu Thr Arg Trp
        35                  40                  45
Thr Gly Met Ile Ile Gly Pro Pro Arg Thr Ile Tyr Glu Asn Arg Ile
    50                  55                  60
Tyr Ser Leu Lys Ile Glu Cys Gly Pro Lys Tyr Pro Glu Ala Pro Pro
65                  70                  75                  80
Phe Val Arg Phe Val Thr Lys Ile Asn Met Asn Gly Val Asn Ser Ser
                85                  90                  95
Asn Gly Val Val Asp Pro Arg Ala Ile Ser Val Leu Ala Lys Trp Gln
            100                 105                 110
Asn Ser Tyr Ser Ile Lys Val Val Leu Gln Glu Leu Arg Arg Leu Met
        115                 120                 125
Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys
    130                 135                 140
Tyr Ser Asn
145
```

FIG.7A

```
atggcacagt tagctggggt ctag   24
```

```
Met Ala Gln Leu Ala Gly Val
 1               5
```

FIG.7B

```
atgacgaaga catga   15
```

```
Met Thr Lys Thr
 1
```

FIG.7C

```
atgacactta caagatggac agggatgata attgggcctc caagaacaat ttatgaaaac   60
cgaatataca gccttaaaat agaatgtgga cctaaatacc cagaagcacc cccctttgta  120
agatttgtaa caaaaattaa tatgaatgga gtaaatagtt ctaatggagt ggtggaccca  180
agagccatat cagtgctagc aaaatggcag aattcatata gcatcaaagt tgtcctgcaa  240
gagcttcggc gcctaatgat gtctaaagaa aatatgaaac tccctcagcc gcccgaagga  300
cagtgttaca gcaattaa                                                318
```

```
Met Thr Leu Thr Arg Trp Thr Gly Met Ile Ile Gly Pro Pro Arg Thr
 1               5                  10                  15
Ile Tyr Glu Asn Arg Ile Tyr Ser Leu Lys Ile Glu Cys Gly Pro Lys
            20                  25                  30
Tyr Pro Glu Ala Pro Pro Phe Val Arg Phe Val Thr Lys Ile Asn Met
        35                  40                  45
Asn Gly Val Asn Ser Ser Asn Gly Val Val Asp Pro Arg Ala Ile Ser
    50                  55                  60
Val Leu Ala Lys Trp Gln Asn Ser Tyr Ser Ile Lys Val Val Leu Gln
65                  70                  75                  80
Glu Leu Arg Arg Leu Met Met Ser Lys Glu Asn Met Lys Leu Pro Gln
                85                  90                  95
Pro Pro Glu Gly Gln Cys Tyr Ser Asn
            100                 105
```

FIG.7D

```
atggacaggg atgataattg ggcctccaag aacaatttat ga   42
```

```
Met Asp Arg Asp Asp Asn Trp Ala Ser Lys Asn Asn Leu
 1               5                  10
```

FIG.7E

```
atgataattg ggcctccaag aacaatttat gaaaaccgaa tatacagcct taaaatagaa   60
tgtggaccta aatacccaga agcacccccc tttgtaagat ttgtaacaaa aattaatatg  120
aatggagtaa atagttctaa tggagtggtg gacccaagag ccatatcagt gctagcaaaa  180
tggcagaatt catatagcat caaagttgtc ctgcaagagc ttcggcgcct aatgatgtct  240
aaagaaaata tgaaactccc tcagccgccc gaaggacagt gttacagcaa ttaa        294

Met Ile Ile Gly Pro Pro Arg Thr Ile Tyr Glu Asn Arg Ile Tyr Ser
 1               5                  10                  15
Leu Lys Ile Glu Cys Gly Pro Lys Tyr Pro Glu Ala Pro Pro Phe Val
            20                  25                  30
Arg Phe Val Thr Lys Ile Asn Met Asn Gly Val Asn Ser Ser Asn Gly
        35                  40                  45
Val Val Asp Pro Arg Ala Ile Ser Val Leu Ala Lys Trp Gln Asn Ser
    50                  55                  60
Tyr Ser Ile Lys Val Val Leu Gln Glu Leu Arg Arg Leu Met Met Ser
65                  70                  75                  80
Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys Tyr Ser
                85                  90                  95
Asn
```

FIG.7F

```
atgaaaaccg aatatacagc cttaaaatag   30

Met Lys Thr Glu Tyr Thr Ala Leu Lys
 1               5
```

FIG.7G

```
atgtggacct aa   12

Met Trp Thr
 1
```

FIG.7H

```
atgaatggag taaatagttc taatggagtg gtggacccaa gagccatatc agtgctagca   60
aaatggcaga attcatatag catcaaagtt gtcctgcaag agcttcggcg cctaatgatg  120
tctaaagaaa atatgaaact ccctcagccg cccgaaggac agtgttacag caattaa     177

Met Asn Gly Val Asn Ser Ser Asn Gly Val Val Asp Pro Arg Ala Ile
 1               5                  10                  15
Ser Val Leu Ala Lys Trp Gln Asn Ser Tyr Ser Ile Lys Val Val Leu
            20                  25                  30
Gln Glu Leu Arg Arg Leu Met Met Ser Lys Glu Asn Met Lys Leu Pro
        35                  40                  45
Gln Pro Pro Glu Gly Gln Cys Tyr Ser Asn
    50                  55
```

FIG.7I

```
atggagtggt ggacccaaga gccatatcag tgctag   36

Met Glu Trp Trp Thr Gln Glu Pro Tyr Gln Cys
 1               5                  10
```

FIG.7J

```
atggcagaat tcatatag   18

Met Ala Glu Phe Ile
 1               5
 2
```

FIG.7K

```
atgatgtcta aagaaaatat gaaactccct cagccgcccg aaggacagtg ttacagcaat   60
taa                                                                 63

Met Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln
 1               5                  10                  15
Cys Tyr Ser Asn
            20
```

FIG.7L

atgtctaaag aaaatatgaa actccctcag ccgcccgaag gacagtgtta cagcaattaa 60

Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys
1 5 10 15
Tyr Ser Asn

FIG.7M

atgaaactcc ctcagccgcc cgaaggacag tgttacagca attaa 45

Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys Tyr Ser Asn
1 5 10

FIG.7N

atgatactaa ttttttcgtc catttga 27

Met Ile Leu Ile Phe Ser Ser Ile
1 5

FIG.7O

atgccccttc caaaccatca tcctgtcccc acgctcctcc actcccgccc ttggccgaag 60
catagattgt aa 72

Met Pro Leu Pro Asn His His Pro Val Pro Thr Leu Leu His Ser Arg
1 5 10 15
Pro Trp Pro Lys His Arg Leu
20

FIG.7P

atgacacttc cttgctttgg ccagaagcca tcaggtaagg ttggaaagag cctctga 57

Met Thr Leu Pro Cys Phe Gly Gln Lys Pro Ser Gly Lys Val Gly Lys
1 5 10 15
Ser Leu

FIG.7Q

atgaatattg ggtcctcagc cctgccaccc tctgctgtca tcagctga 48

Met Asn Ile Gly Ser Ser Ala Leu Pro Pro Ser Ala Val Ile Ser
1 5 10 15

FIG.7R

```
atgcattgtt tttag   15

Met His Cys Phe
 1
```

FIG.7S

```
atgaagatac ttgtaagcac acatgatccc tctgaattgt tttactttcc tgtaactgct   60
tttgctttta aaaattga                                                  78

Met Lys Ile Leu Val Ser Thr His Asp Pro Ser Glu Leu Phe Tyr Phe
 1               5                  10                  15
Pro Val Thr Ala Phe Ala Phe Lys Asn
            20                  25
```

FIG.7T

```
atgatccctc tgaattgttt tactttcctg taa   33

Met Ile Pro Leu Asn Cys Phe Thr Phe Leu
 1               5                  10
```

FIG.7U

```
atgccttggt tttggtgctg ctgctgcttc ccaagatcct cagcagggat taagaaggaa   60
cccggtgtgc acagcagatc cccgaaattg gtgggcttga cctcctggca aattgctgcg  120
tctttccact tgctgttcag gaccactaaa tgctga                             156

Met Pro Trp Phe Trp Cys Cys Cys Cys Phe Pro Arg Ser Ser Ala Gly
 1               5                  10                  15
Ile Lys Lys Glu Pro Gly Val His Ser Arg Ser Pro Lys Leu Val Gly
            20                  25                  30
Leu Thr Ser Trp Gln Ile Ala Ala Ser Phe His Leu Leu Phe Arg Thr
        35                  40                  45
Thr Lys Cys
    50
```

FIG.7V

```
atgctgaaat gtggatgcat accgaaataa   30

Met Leu Lys Cys Gly Cys Ile Pro Lys
 1               5
```

FIG.7W

```
atgtggatgc ataccgaaat aaaagcaatt cattgtgtac taaaggtttt tttttttttt   60
ttaatttag                                                           69

Met Trp Met His Thr Glu Ile Lys Ala Ile His Cys Val Leu Lys Val
 1               5                  10                  15
Phe Phe Phe Phe Leu Ile
            20
```

FIG.7X

```
atgcataccg aaataaaagc aattcattgt gtactaaagg tttttttttt tttttaatt   60
tag                                                                63

Met His Thr Glu Ile Lys Ala Ile His Cys Val Leu Lys Val Phe Phe
 1               5                  10                  15
Phe Phe Leu Ile
            20
```

FIG.7Y

```
atgtttccat taatcttttt ctgggggaa aaccttagtt ctaaggattt aacatcctgt   60
aagtga                                                             66

Met Phe Pro Leu Ile Phe Phe Trp Gly Glu Asn Leu Ser Ser Lys Asp
 1               5                  10                  15
Leu Thr Ser Cys Lys
            20
```

FIG.7Z

atgtgtgtgt gtgtgtgtat gtgtgtgttt ctgagtaagt attga 45

Met Cys Val Cys Val Cys Met Cys Val Phe Leu Ser Lys Tyr
1 5 10

FIG.8A

atgtgtgtgt ttctgagtaa gtattga 27

Met Cys Val Phe Leu Ser Lys Tyr
1 5

FIG.8B

atgacgaaat ga 12

Met Thr Lys
1

FIG.8C

atgagatcaa taggaaatgt gctttttgag gaaattttat tttag 45

Met Arg Ser Ile Gly Asn Val Leu Phe Glu Glu Ile Leu Phe
1 5 10

FIG.8D

atgtgctttt tgaggaaatt ttattttagt accaaatgtt gccagtga 48

Met Cys Phe Leu Arg Lys Phe Tyr Phe Ser Thr Lys Cys Cys Gln
1 5 10 15

FIG.8E

atgttgccag tgacaatctt cagttaa 27

Met Leu Pro Val Thr Ile Phe Ser
1 5

FIG.8F

atgaataagc agcatttttc attgcactta aaaatgtaa 39

Met Asn Lys Gln His Phe Ser Leu His Leu Lys Met
1 5 10

FIG.8G

atgccactaa tctgtaacat tttaccagtt cagatgcctg taatgtgtga ctttatgtgt 60
gtctgtgttg ttttgaagag aataaaggaa ataatacttt gcaaactgtt taaacaagtg 120
tttaaacttc tattggcaac atttattggg ctaagcagtt attga 165

Met Pro Leu Ile Cys Asn Ile Leu Pro Val Gln Met Pro Val Met Cys
1 5 10 15
Asp Phe Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu Ile Ile
20 25 30
Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala Thr Phe
35 40 45
Ile Gly Leu Ser Ser Tyr
50

FIG.8H

atgcctgtaa tgtgtgactt tatgtgtgtc tgtgttgttt tgaagagaat aaaggaaata 60
atactttgca aactgtttaa acaagtgttt aaacttctat tggcaacatt tattgggcta 120
agcagttatt ga 132

Met Pro Val Met Cys Asp Phe Met Cys Val Cys Val Val Leu Lys Arg
1 5 10 15
Ile Lys Glu Ile Ile Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu
20 25 30
Leu Leu Ala Thr Phe Ile Gly Leu Ser Ser Tyr
35 40

FIG.8I

```
atgtgtgact ttatgtgtgt ctgtgttgtt ttgaagagaa taaaggaaat aatactttgc   60
aaactgttta aacaagtgtt taaacttcta ttggcaacat ttattgggct aagcagttat  120
tga                                                                123

Met Cys Asp Phe Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu
 1               5                  10                  15
Ile Ile Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala
            20                  25                  30
Thr Phe Ile Gly Leu Ser Ser Tyr
        35                  40
```

FIG.8J

```
atgtgtgtct gtgttgtttt gaagagaata aaggaaataa tactttgcaa actgtttaaa   60
caagtgttta aacttctatt ggcaacattt attgggctaa gcagttattg a           111

Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu Ile Ile Leu Cys
 1               5                  10                  15
Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala Thr Phe Ile Gly
            20                  25                  30
Leu Ser Ser Tyr
        35
```

FIG.8K

```
atgtgtgatg aagcaaaatg tataaagtat gaaatattat acttttaccc tggataa   57

Met Cys Asp Glu Ala Lys Cys Ile Lys Tyr Glu Ile Leu Tyr Phe Tyr
 1               5                  10                  15
Pro Gly
```

FIG.8L

```
atgaagcaaa atgtataa   18

Met Lys Gln Asn Val
 1               5
```

FIG.8M

```
atgtataaag tatga   15

Met Tyr Lys Val
 1
```

FIG.8N

```
atgaaatatt atacttttac cctggataat tattcaggac cccagttggc ccaaataggt   60
gcaattttta atcctttgaa attagccagc cagacctaa                          99

Met Lys Tyr Tyr Thr Phe Thr Leu Asp Asn Tyr Ser Gly Pro Gln Leu
 1               5                  10                  15
Ala Gln Ile Gly Ala Ile Phe Asn Pro Leu Lys Leu Ala Ser Gln Thr
            20                  25                  30
```

FIG.8O

```
atgctaaggt aa   12

Met Leu Arg
 1
```

FIG.8P

```
atgtatctat ttctgtcagg aatgatattt ccaaatgaaa atgtaaagaa cattgggaaa   60
taa                                                                 63

Met Tyr Leu Phe Leu Ser Gly Met Ile Phe Pro Asn Glu Asn Val Lys
 1               5                  10                  15
Asn Ile Gly Lys
            20
```

FIG.8Q

```
atgatatttc caaatgaaaa tgtaaagaac attgggaaat aa   42

Met Ile Phe Pro Asn Glu Asn Val Lys Asn Ile Gly Lys
 1               5                  10
```

FIG.8R

```
atgaaaatgt aa   12

Met Lys Met
 1
```

FIG.8S

atgaagagta acagtgtaga ccagactgcc tctctcagat atgtgcctga tattttgtgg 60
atacctcccc tgcactggca aaacactatg cttttgggtg ttagactgaa atattttaag 120
agtatttaa 129

Met Lys Ser Asn Ser Val Asp Gln Thr Ala Ser Leu Arg Tyr Val Pro
1 5 10 15
Asp Ile Leu Trp Ile Pro Pro Leu His Trp Gln Asn Thr Met Leu Leu
20 25 30
Gly Val Arg Leu Lys Tyr Phe Lys Ser Ile
35 40

FIG.9A

atgtgcctga tattttgtgg atacctcccc tgcactggca aaacactatg cttttgggtg 60
ttagactga 69

Met Cys Leu Ile Phe Cys Gly Tyr Leu Pro Cys Thr Gly Lys Thr Leu
1 5 10 15
Cys Phe Trp Val Leu Asp
20

FIG.9B

atgcttttgg gtgttagact gaaatatttt aagagtattt aa 42

Met Leu Leu Gly Val Arg Leu Lys Tyr Phe Lys Ser Ile
1 5 10

FIG.9C

atggaaatgt atcttatgaa tagagacata ttaaaataa 39

Met Glu Met Tyr Leu Met Asn Arg Asp Ile Leu Lys
1 5 10

FIG.9D

atgtatctta tgaatagaga catattaaaa taa 33

Met Tyr Leu Met Asn Arg Asp Ile Leu Lys
1 5 10

FIG.9E

atgaatagag acatattaaa ataa 24

Met Asn Arg Asp Ile Leu Lys
1 5

FIG.9F

atgtttacat cttag 15

Met Phe Thr Ser
1

FIG.9G

atggtttctg gagacaaata a 21

Met Val Ser Gly Asp Lys
1 5

FIG.9H

atgatttatt ttttgactaa atgtgcaatt tcttatcact ag 42

Met Ile Tyr Phe Leu Thr Lys Cys Ala Ile Ser Tyr His
1 5 10

FIG.9I

atgtgcaatt tcttatcact agataacttt cagtatcagt ggtggttact tattacttaa 60

Met Cys Asn Phe Leu Ser Leu Asp Asn Phe Gln Tyr Gln Trp Trp Leu
1 5 10 15
Leu Ile Thr

FIG.9J

atgtcgactt gctaa 15

Met Ser Thr Cys
1

FIG.9K

atgtcttttt ttttagtgtc ccaaagatat cttagataa 39

Met Ser Phe Phe Leu Val Ser Gln Arg Tyr Leu Arg
1 5 10

FIG.9L

atgaggcaac attttcttga gataattacc caagtttcat ccatgttgaa tggtacaaaa 60
tatttctgtg aaactaacag gaagatattt tcagataact ag 102

Met Arg Gln His Phe Leu Glu Ile Ile Thr Gln Val Ser Ser Met Leu
1 5 10 15
Asn Gly Thr Lys Tyr Phe Cys Glu Thr Asn Arg Lys Ile Phe Ser Asp
20 25 30
Asn

FIG.9M

atgttgaatg gtacaaaata tttctgtgaa actaacagga agatattttc agataactag 60

Met Leu Asn Gly Thr Lys Tyr Phe Cys Glu Thr Asn Arg Lys Ile Phe
1 5 10 15
Ser Asp Asn

FIG.9N

atggtacaaa atatttctgt gaaactaaca ggaagatatt ttcagataac taggataact 60
tgttgctttg ttacccagcc taattga 87

Met Val Gln Asn Ile Ser Val Lys Leu Thr Gly Arg Tyr Phe Gln Ile
1 5 10 15
Thr Arg Ile Thr Cys Cys Phe Val Thr Gln Pro Asn
20 25

FIG.9O

atgcttgttt caaagaacca acagaaaaaa aagctaagaa aactgagaac taacattaaa 60
aaaattaaat ttagaataag aatgatttct ttaatttgtc cttttttct ttggtctaaa 120
acattattaa atttttgtaa atattttgat ttaatgtgtc ttagatcctc attattttaa 180

Met Leu Val Ser Lys Asn Gln Gln Lys Lys Lys Leu Arg Lys Leu Arg
1 5 10 15
Thr Asn Ile Lys Lys Ile Lys Phe Arg Ile Arg Met Ile Ser Leu Ile
20 25 30
Cys Pro Phe Phe Leu Trp Ser Lys Thr Leu Leu Asn Phe Cys Lys Tyr
35 40 45
Phe Asp Leu Met Cys Leu Arg Ser Ser Leu Phe
50 55

FIG.9P

atgatttctt taatttgtcc ttttttctt tggtctaaaa cattattaaa tttttgtaaa 60
tattttgatt taatgtgtct tagatcctca ttattttaa 99

Met Ile Ser Leu Ile Cys Pro Phe Phe Leu Trp Ser Lys Thr Leu Leu
1 5 10 15
Asn Phe Cys Lys Tyr Phe Asp Leu Met Cys Leu Arg Ser Ser Leu Phe
20 25 30

FIG.9Q

atgtgtctta gatcctcatt attttaa 27

Met Cys Leu Arg Ser Ser Leu Phe
1 5

FIG.9R

atgctaatat gtaaagttca tgccatccag gcatttaaga gcgatcctca tcccttcagc 60
aatatgtatt tgagttcaca ctatttctgt tttacagcag ttttgaaaaa cacatactat 120
gccaccaatt gtcatattat ttttagatga 150

Met Leu Ile Cys Lys Val His Ala Ile Gln Ala Phe Lys Ser Asp Pro
1 5 10 15
His Pro Phe Ser Asn Met Tyr Leu Ser Ser His Tyr Phe Cys Phe Thr
20 25 30
Ala Val Leu Lys Asn Thr Tyr Tyr Ala Thr Asn Cys His Ile Ile Phe
35 40 45
Arg

FIG.9S

atgccatcca ggcatttaag agcgatcctc atcccttcag caatatgtat ttga 54

Met Pro Ser Arg His Leu Arg Ala Ile Leu Ile Pro Ser Ala Ile Cys
1 5 10 15
Ile

FIG.9T

atgtatttga gttcacacta tttctgtttt acagcagttt tgaaaaacac atactatgcc 60
accaattgtc atattatttt tagatga 87

Met Tyr Leu Ser Ser His Tyr Phe Cys Phe Thr Ala Val Leu Lys Asn
1 5 10 15
Thr Tyr Tyr Ala Thr Asn Cys His Ile Ile Phe Arg
20 25

FIG.9U

atgccaccaa ttgtcatatt atttttagat gatgtaacat ag 42

Met Pro Pro Ile Val Ile Leu Phe Leu Asp Asp Val Thr
1 5 10

FIG.9V

atgcctaata cttag 15

Met Pro Asn Thr
1

FIG.9W

atgtcacgag atcattttta cattaaacgt gaaaaaaaat caaaaaaaaa aaaaaaaaaa 60
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 120
aaaaaaaaaa agggcggcc 139

Met Ser Arg Asp His Phe Tyr Ile Lys Arg Glu Lys Lys Ser Lys Lys
1 5 10 15
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
20 25 30
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Gly Gly
35 40 45

FIG.9X

atgagccggc aggtggtccg ctccagcaag ttccgccacg tgtttggaca gccggccaag 60
gccgaccagt gctatgaaga tgtgcgcgtc tcacagacca cctgggacag tggcttctgt 120
gctgtcaacc ctaagtttgt ggccctgatc tgtgaggcca gcgggggagg ggccttcctg 180
gtgctgcccc tgggcaagac tggacgtgtg gacaagaatg cgcccacggt ctgtggccac 240
acagcccctg tgctagacat cgcctggtgc ccgcacaatg acaacgtcat tgccagtggc 300
tccgaggact gcacagtcat ggtgtgggag atcccagatg gggggcctgat gctgcccctg 360
cgggagcccg tcgtcaccct ggagggccac accaagcgtg tgggcattgt ggcctggcac 420
accacagccc agaacgtgct gctcagtgca ggttgtgaca acgtgatcat ggtgtgggac 480
gtgggcactg gggcggccat gctgacactg ggcccagagg tgcacccaga cacgatctac 540
agtgtggact ggagccgaga tggaggcctc atttgtacct cctgccgtga caagcgcgtg 600
cgcatcatcg agccccgcaa aggcactgtc gtagctgaga aggaccgtcc ccacgagggg 660
acccggcccg tgcgtgcagt gttcgtgtcg gaggggaaga tcctgaccac gggcttcagc 720
cgcatgagtg agcggcaggt ggcgctgtgg gacacaaagc acctggagga gccgctgtcc 780
ctgcaggagc tggacaccag cagcggtgtc ctgctgccct tctttgaccc tgacaccaac 840
atcgtctacc tctgtggcaa gggtgacagc tcaatccggt actttgagat cacttccgag 900
gccccttttcc tgcactatct ctccatgttc agttccaagg agtcccagcg gggcatgggc 960
tacatgccca aacgtggcct ggaggtgaac aagtgtgaga tcgccaggtt ctacaagctg 1020
cacgagcgga ggtgtgagcc cattgccatg acagtgcctc gaaagtcgga cctgttccag 1080
gaggacctgt acccacccac cgcagggccc gaccctgccc tcacggctga ggagtggctg 1140
gggggtcggg atgctgggcc cctcctcatc tccctcaagg atggctacgt acccccaaag 1200
agccgggagc tgagggtcaa ccggggcctg gacaccgggc gcaggagggc agcaccagag 1260
gccagtggca ctcccagctc ggatgccgtg tctcggctgg aggaggagat gcggaagctc 1320
caggccacgg tgcaggagct ccagaagcgc ttggacaggc tggaggagac agtccaggcc 1380
aagtag 1386

Met Ser Arg Gln Val Val Arg Ser Ser Lys Phe Arg His Val Phe Gly
1 5 10 15
Gln Pro Ala Lys Ala Asp Gln Cys Tyr Glu Asp Val Arg Val Ser Gln
20 25 30
Thr Thr Trp Asp Ser Gly Phe Cys Ala Val Asn Pro Lys Phe Val Ala
35 40 45
Leu Ile Cys Glu Ala Ser Gly Gly Gly Ala Phe Leu Val Leu Pro Leu
50 55 60
Gly Lys Thr Gly Arg Val Asp Lys Asn Ala Pro Thr Val Cys Gly His
65 70 75 80
Thr Ala Pro Val Leu Asp Ile Ala Trp Cys Pro His Asn Asp Asn Val
85 90 95
Ile Ala Ser Gly Ser Glu Asp Cys Thr Val Met Val Trp Glu Ile Pro
100 105 110
Asp Gly Gly Leu Met Leu Pro Leu Arg Glu Pro Val Val Thr Leu Glu
115 120 125
Gly His Thr Lys Arg Val Gly Ile Val Ala Trp His Thr Thr Ala Gln
130 135 140

FIG.10A-1

Asn Val Leu Leu Ser Ala Gly Cys Asp Asn Val Ile Met Val Trp Asp
145 150 155 160
Val Gly Thr Gly Ala Ala Met Leu Thr Leu Gly Pro Glu Val His Pro
165 170 175
Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys
180 185 190
Thr Ser Cys Arg Asp Lys Arg Val Arg Ile Ile Glu Pro Arg Lys Gly
195 200 205
Thr Val Val Ala Glu Lys Asp Arg Pro His Glu Gly Thr Arg Pro Val
210 215 220
Arg Ala Val Phe Val Ser Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser
225 230 235 240
Arg Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu
245 250 255
Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu
260 265 270
Pro Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly
275 280 285
Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu
290 295 300
His Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly
305 310 315 320
Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg
325 330 335
Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val
340 345 350
Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala
355 360 365
Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp
370 375 380
Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys
385 390 395 400
Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg
405 410 415
Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg
420 425 430
Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln
435 440 445
Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
450 455 460

FIG.10A-2

```
atgaagatgt gcgcgtctca cagaccacct gggacagtgg cttctgtgct gtcaacccta   60
agtttgtggc cctga                                                    75

Met Lys Met Cys Ala Ser His Arg Pro Pro Gly Thr Val Ala Ser Val
 1               5                  10                  15
Leu Ser Thr Leu Ser Leu Trp Pro
            20
```

FIG.10B

```
atgtgcgcgt ctcacagacc acctgggaca gtggcttctg tgctgtcaac cctaagtttg   60
tggccctga                                                           69

Met Cys Ala Ser His Arg Pro Pro Gly Thr Val Ala Ser Val Leu Ser
 1               5                  10                  15
Thr Leu Ser Leu Trp Pro
            20
```

FIG.10C

```
atgcgcccac ggtctgtggc cacacagccc ctgtgctag   39

Met Arg Pro Arg Ser Val Ala Thr Gln Pro Leu Cys
 1               5                  10
```

FIG.10D

```
atgacaacgt cattgccagt ggctccgagg actgcacagt catggtgtgg gagatcccag   60
atgggggcct ga                                                       72

Met Thr Thr Ser Leu Pro Val Ala Pro Arg Thr Ala Gln Ser Trp Cys
 1               5                  10                  15
Gly Arg Ser Gln Met Gly Ala
            20
```

FIG.10E

```
atggtgtggg agatcccaga tgggggcctg atgctgcccc tgcgggagcc cgtcgtcacc   60
ctggagggcc acaccaagcg tgtgggcatt gtggcctggc acaccacagc ccagaacgtg  120
ctgctcagtg caggttgtga caacgtgatc atggtgtggg acgtgggcac tggggcggcc  180
atgctgacac tgggcccaga ggtgcaccca gacacgatct acagtgtgga ctggagccga  240
gatggaggcc tcatttgtac ctcctgccgt gacaagcgcg tgcgcatcat cgagccccgc  300
aaaggcactg tcgtagctga gaaggaccgt ccccacgagg ggacccggcc cgtgcgtgca  360
gtgttcgtgt cggaggggaa gatcctgacc acgggcttca gccgcatgag tgagcggcag  420
gtggcgctgt gggacacaaa gcacctggag gagccgctgt ccctgcagga gctggacacc  480
agcagcggtg tcctgctgcc cttctttgac cctgacacca acatcgtcta cctctgtggc  540
aagggtgaca gctcaatccg gtactttgag atcacttccg aggccccttt cctgcactat  600
ctctccatgt tcagttccaa ggagtcccag cggggcatgg gctacatgcc caaacgtggc  660
ctggaggtga acaagtgtga gatcgccagg ttctacaagc tgcacgagcg gaggtgtgag  720
cccattgcca tgacagtgcc tcgaaagtcg gacctgttcc aggaggacct gtacccaccc  780
accgcagggc ccgaccctgc cctcacggct gaggagtggc tgggggtcg ggatgctggg  840
cccctcctca tctccctcaa ggatggctac gtacccccaa agagccggga gctgagggtc  900
aaccggggcc tggacaccgg gcgcaggagg gcagcaccag aggccagtgg cactcccagc  960
tcggatgccg tgtctcggct ggaggaggag atgcggaagc tccaggccac ggtgcaggag 1020
ctccagaagc gcttggacag gctggaggag acagtccagg ccaagtag              1068
```

```
Met Val Trp Glu Ile Pro Asp Gly Gly Leu Met Leu Pro Leu Arg Glu
 1               5                  10                  15
Pro Val Val Thr Leu Glu Gly His Thr Lys Arg Val Gly Ile Val Ala
            20                  25                  30
Trp His Thr Thr Ala Gln Asn Val Leu Leu Ser Ala Gly Cys Asp Asn
        35                  40                  45
Val Ile Met Val Trp Asp Val Gly Thr Gly Ala Ala Met Leu Thr Leu
    50                  55                  60
Gly Pro Glu Val His Pro Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg
65                  70                  75                  80
Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys Arg Val Arg Ile
                85                  90                  95
Ile Glu Pro Arg Lys Gly Thr Val Val Ala Glu Lys Asp Arg Pro His
            100                 105                 110
Glu Gly Thr Arg Pro Val Arg Ala Val Phe Val Ser Glu Gly Lys Ile
        115                 120                 125
Leu Thr Thr Gly Phe Ser Arg Met Ser Glu Arg Gln Val Ala Leu Trp
    130                 135                 140
Asp Thr Lys His Leu Glu Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr
145                 150                 155                 160
Ser Ser Gly Val Leu Leu Pro Phe Phe Asp Pro Asp Thr Asn Ile Val
                165                 170                 175
```

FIG.10F-1

```
Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr
            180                 185                 190
Ser Glu Ala Pro Phe Leu His Tyr Leu Ser Met Phe Ser Ser Lys Glu
        195                 200                 205
Ser Gln Arg Gly Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn
    210                 215                 220
Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu
225                 230                 235                 240
Pro Ile Ala Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp
                245                 250                 255
Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu
            260                 265                 270
Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp
        275                 280                 285
Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu
    290                 295                 300
Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser
305                 310                 315                 320
Ser Asp Ala Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala
                325                 330                 335
Thr Val Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val
            340                 345                 350
Gln Ala Lys
        355
```

FIG.10F-2

```
atgggggcct ga    12

Met Gly Ala
 1
```

FIG.10G

```
atgctgcccc tgcgggagcc cgtcgtcacc ctggagggcc acaccaagcg tgtgggcatt   60
gtggcctggc acaccacagc ccagaacgtg ctgctcagtg caggttgtga caacgtgatc  120
atggtgtggg acgtgggcac tggggcggcc atgctgacac tgggcccaga ggtgcaccca  180
gacacgatct acagtgtgga ctggagccga gatggaggcc tcatttgtac ctcctgccgt  240
gacaagcgcg tgcgcatcat cgagccccgc aaaggcactg tcgtagctga gaaggaccgt  300
ccccacgagg ggacccggcc cgtgcgtgca gtgttcgtgt cggaggggaa gatcctgacc  360
acgggcttca gccgcatgag tgagcggcag gtggcgctgt gggacacaaa gcacctggag  420
gagccgctgt ccctgcagga gctggacacc agcagcggtg tcctgctgcc cttctttgac  480
cctgacacca acatcgtcta cctctgtggc aagggtgaca gctcaatccg gtactttgag  540
atcacttccg aggccccttt cctgcactat ctctccatgt tcagttccaa ggagtcccag  600
cggggcatgg gctacatgcc caaacgtggc ctggaggtga acaagtgtga gatcgccagg  660
ttctacaagc tgcacgagcg gaggtgtgag cccattgcca tgacagtgcc tcgaaagtcg  720
gacctgttcc aggaggacct gtacccaccc accgcagggc ccgaccctgc cctcacggct  780
gaggagtggc tgggggtcg ggatgctggg cccctcctca tctccctcaa ggatggctac  840
gtacccccaa agagccggga gctgagggtc aaccggggcc tggacaccgg gcgcaggagg  900
gcagcaccag aggccagtgg cactcccagc tcggatgccg tgtctcggct ggaggaggag  960
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag 1020
acagtccagg ccaagtag                                              1038
```

```
Met Leu Pro Leu Arg Glu Pro Val Val Thr Leu Glu Gly His Thr Lys
 1               5                  10                  15
Arg Val Gly Ile Val Ala Trp His Thr Thr Ala Gln Asn Val Leu Leu
            20                  25                  30
Ser Ala Gly Cys Asp Asn Val Ile Met Val Trp Asp Val Gly Thr Gly
        35                  40                  45
Ala Ala Met Leu Thr Leu Gly Pro Glu Val His Pro Asp Thr Ile Tyr
    50                  55                  60
Ser Val Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg
65                  70                  75                  80
Asp Lys Arg Val Arg Ile Ile Glu Pro Arg Lys Gly Thr Val Val Ala
                85                  90                  95
Glu Lys Asp Arg Pro His Glu Gly Thr Arg Pro Val Arg Ala Val Phe
            100                 105                 110
Val Ser Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser Arg Met Ser Glu
        115                 120                 125
Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu Pro Leu Ser
    130                 135                 140
Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro Phe Phe Asp
145                 150                 155                 160
Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile
                165                 170                 175
```

FIG.10H-1

```
Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His Tyr Leu Ser
            180                 185                 190
Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys
        195                 200                 205
Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu
    210                 215                 220
His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser
225                 230                 235                 240
Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro
                245                 250                 255
Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu
            260                 265                 270
Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu
        275                 280                 285
Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu
    290                 295                 300
Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu
305                 310                 315                 320
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
                325                 330                 335
Arg Leu Glu Glu Thr Val Gln Ala Lys
            340                 345
```

FIG.10H-2

atggtgtggg acgtgggcac tggggcggcc atgctgacac tgggcccaga ggtgcaccca 60
gacacgatct acagtgtgga ctggagccga gatggaggcc tcatttgtac ctcctgccgt 120
gacaagcgcg tgcgcatcat cgagccccgc aaaggcactg tcgtagctga gaaggaccgt 180
ccccacgagg ggacccggcc cgtgcgtgca gtgttcgtgt cggaggggaa gatcctgacc 240
acgggcttca gccgcatgag tgagcggcag gtggcgctgt gggacacaaa gcacctggag 300
gagccgctgt ccctgcagga gctggacacc agcagcggtg tcctgctgcc cttctttgac 360
cctgacacca acatcgtcta cctctgtggc aagggtgaca gctcaatccg gtactttgag 420
atcacttccg aggccccttt cctgcactat ctctccatgt tcagttccaa ggagtcccag 480
cggggcatgg gctacatgcc caaacgtggc ctggaggtga acaagtgtga gatcgccagg 540
ttctacaagc tgcacgagcg gaggtgtgag cccattgcca tgacagtgcc tcgaaagtcg 600
gacctgttcc aggaggacct gtacccaccc accgcagggc ccgaccctgc cctcacggct 660
gaggagtggc tgggggggtcg ggatgctggg cccctcctca tctccctcaa ggatggctac 720
gtacccccaa agagccggga gctgagggtc aaccggggcc tggacaccgg gcgcaggagg 780
gcagcaccag aggccagtgg cactcccagc tcggatgccg tgtctcggct ggaggaggag 840
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag 900
acagtccagg ccaagtag 918

Met Val Trp Asp Val Gly Thr Gly Ala Ala Met Leu Thr Leu Gly Pro
1 5 10 15
Glu Val His Pro Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg Asp Gly
20 25 30
Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys Arg Val Arg Ile Ile Glu
35 40 45
Pro Arg Lys Gly Thr Val Val Ala Glu Lys Asp Arg Pro His Glu Gly
50 55 60
Thr Arg Pro Val Arg Ala Val Phe Val Ser Glu Gly Lys Ile Leu Thr
65 70 75 80
Thr Gly Phe Ser Arg Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr
85 90 95
Lys His Leu Glu Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser
100 105 110
Gly Val Leu Leu Pro Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu
115 120 125
Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu
130 135 140
Ala Pro Phe Leu His Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln
145 150 155 160
Arg Gly Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys
165 170 175
Glu Ile Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile
180 185 190

FIG.10I-1

```
Ala Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr
        195                 200                 205
Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu
    210                 215                 220
Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr
225                 230                 235                 240
Val Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr
                245                 250                 255
Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp
            260                 265                 270
Ala Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val
        275                 280                 285
Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala
    290                 295                 300
Lys
305
```

FIG.10I-2

```
atgctgacac tgggcccaga ggtgcaccca gacacgatct acagtgtgga ctggagccga   60
gatggaggcc tcatttgtac ctcctgccgt gacaagcgcg tgcgcatcat cgagccccgc  120
aaaggcactg tcgtagctga gaaggaccgt ccccacgagg ggacccggcc cgtgcgtgca  180
gtgttcgtgt cggaggggaa gatcctgacc acgggcttca gccgcatgag tgagcggcag  240
gtggcgctgt gggacacaaa gcacctggag gagccgctgt ccctgcagga gctggacacc  300
agcagcggtg tcctgctgcc cttctttgac cctgacacca acatcgtcta cctctgtggc  360
aagggtgaca gctcaatccg gtactttgag atcacttccg aggccccttt cctgcactat  420
ctctccatgt tcagttccaa ggagtcccag cggggcatgg gctacatgcc caaacgtggc  480
ctggaggtga acaagtgtga gatcgccagg ttctacaagc tgcacgagcg gaggtgtgag  540
cccattgcca tgacagtgcc tcgaaagtcg gacctgttcc aggaggacct gtacccaccc  600
accgcagggc ccgaccctgc cctcacggct gaggagtggc tgggggggtcg ggatgctggg  660
cccctcctca tctccctcaa ggatggctac gtacccccaa agagccggga gctgagggtc  720
aaccggggcc tggacaccgg gcgcaggagg gcagcaccag aggccagtgg cactcccagc  780
tcggatgccg tgtctcggct ggaggaggag atgcggaagc tccaggccac ggtgcaggag  840
ctccagaagc gcttggacag gctggaggag acagtccagg ccaagtag                888
```

```
Met Leu Thr Leu Gly Pro Glu Val His Pro Asp Thr Ile Tyr Ser Val
 1               5                  10                  15
Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys
            20                  25                  30
Arg Val Arg Ile Ile Glu Pro Arg Lys Gly Thr Val Val Ala Glu Lys
        35                  40                  45
Asp Arg Pro His Glu Gly Thr Arg Pro Val Arg Ala Val Phe Val Ser
    50                  55                  60
Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser Arg Met Ser Glu Arg Gln
65                  70                  75                  80
Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu Pro Leu Ser Leu Gln
                85                  90                  95
Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro Phe Phe Asp Pro Asp
            100                 105                 110
Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr
        115                 120                 125
Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His Tyr Leu Ser Met Phe
    130                 135                 140
Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys Arg Gly
145                 150                 155                 160
Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu His Glu
                165                 170                 175
Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser Asp Leu
            180                 185                 190
Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu
        195                 200                 205
```

FIG.10J-1

Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile
    210                 215                 220
Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu Arg Val
225                 230                 235                 240
Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser
                245                 250                 255
Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu Met Arg
            260                 265                 270
Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu
        275                 280                 285
Glu Glu Thr Val Gln Ala Lys
    290                 295

FIG.10J-2

atggaggcct catttgtacc tcctgccgtg acaagcgcgt gcgcatcatc gagccccgca 60
aaggcactgt cgtag 75

Met Glu Ala Ser Phe Val Pro Pro Ala Val Thr Ser Ala Cys Ala Ser
1               5                   10                  15
Ser Ser Pro Ala Lys Ala Leu Ser
            20

FIG.10K

```
atgagtgagc ggcaggtggc gctgtgggac acaaagcacc tggaggagcc gctgtccctg   60
caggagctgg acaccagcag cggtgtcctg ctgcccttct ttgaccctga caccaacatc  120
gtctacctct gtggcaaggg tgacagctca atccggtact ttgagatcac ttccgaggcc  180
cctttcctgc actatctctc catgttcagt tccaaggagt cccagcgggg catgggctac  240
atgcccaaac gtggcctgga ggtgaacaag tgtgagatcg ccaggttcta caagctgcac  300
gagcggaggt gtgagcccat tgccatgaca gtgcctcgaa agtcggacct gttccaggag  360
gacctgtacc cacccaccgc agggcccgac cctgccctca cggctgagga gtggctgggg  420
ggtcgggatg ctgggcccct cctcatctcc ctcaaggatg gctacgtacc cccaaagagc  480
cgggagctga gggtcaaccg gggcctggac accgggcgca ggagggcagc accagaggcc  540
agtggcactc ccagctcgga tgccgtgtct cggctggagg aggagatgcg gaagctccag  600
gccacggtgc aggagctcca gaagcgcttg gacaggctgg aggagacagt ccaggccaag  660
tag                                                                663

Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu
 1               5                  10                  15
Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro
            20                  25                  30
Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp
        35                  40                  45
Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His
    50                  55                  60
Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr
65                  70                  75                  80
Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe
                85                  90                  95
Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro
            100                 105                 110
Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly
        115                 120                 125
Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala
    130                 135                 140
Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser
145                 150                 155                 160
Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala
                165                 170                 175
Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu
            180                 185                 190
Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys
        195                 200                 205
Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
    210                 215                 220
```

FIG.10L

```
atgttcagtt ccaaggagtc ccagcggggc atgggctaca tgcccaaacg tggcctggag    60
gtgaacaagt gtgagatcgc caggttctac aagctgcacg agcggaggtg tgagcccatt   120
gccatgacag tgcctcgaaa gtcggacctg ttccaggagg acctgtaccc acccaccgca   180
gggcccgacc ctgccctcac ggctgaggag tggctggggg gtcgggatgc tgggcccctc   240
ctcatctccc tcaaggatgg ctacgtaccc ccaaagagcc gggagctgag ggtcaaccgg   300
ggcctggaca ccgggcgcag gagggcagca ccagaggcca gtggcactcc cagctcggat   360
gccgtgtctc ggctggagga ggagatgcgg aagctccagg ccacggtgca ggagctccag   420
aagcgcttgg acaggctgga ggagacagtc caggccaagt ag                      462
```

```
Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys
 1               5                  10                  15
Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu
            20                  25                  30
His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser
        35                  40                  45
Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro
    50                  55                  60
Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu
65                  70                  75                  80
Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu
                85                  90                  95
Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu
            100                 105                 110
Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu
        115                 120                 125
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
    130                 135                 140
Arg Leu Glu Glu Thr Val Gln Ala Lys
145                 150
```

FIG.10M

```
atgggctaca tgcccaaacg tggcctggag gtgaacaagt gtgagatcgc caggttctac    60
aagctgcacg agcggaggtg tgagcccatt gccatgacag tgcctcgaaa gtcggacctg   120
ttccaggagg acctgtaccc acccaccgca gggcccgacc ctgccctcac ggctgaggag   180
tggctggggg gtcgggatgc tgggcccctc ctcatctccc tcaaggatgg ctacgtaccc   240
ccaaagagcc gggagctgag ggtcaaccgg ggcctggaca ccgggcgcag gagggcagca   300
ccagaggcca gtggcactcc cagctcggat gccgtgtctc ggctggagga ggagatgcgg   360
aagctccagg ccacggtgca ggagctccag aagcgcttgg acaggctgga ggagacagtc   420
caggccaagt ag                                                        432
```

```
Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile
1               5                   10                  15
Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met
            20                  25                  30
Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro
        35                  40                  45
Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly
    50                  55                  60
Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro
65                  70                  75                  80
Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg
                85                  90                  95
Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val
            100                 105                 110
Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu
        115                 120                 125
Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
    130                 135                 140
```

FIG.10N

atgcccaaac gtggcctgga ggtgaacaag tgtgagatcg ccaggttcta caagctgcac 60
gagcggaggt gtgagcccat tgccatgaca gtgcctcgaa agtcggacct gttccaggag 120
gacctgtacc cacccaccgc agggcccgac cctgccctca cggctgagga gtggctgggg 180
ggtcgggatg ctgggcccct cctcatctcc ctcaaggatg gctacgtacc cccaaagagc 240
cgggagctga gggtcaaccg gggcctggac accgggcgca ggagggcagc accagaggcc 300
agtggcactc ccagctcgga tgccgtgtct cggctggagg aggagatgcg gaagctccag 360
gccacggtgc aggagctcca gaagcgcttg gacaggctgg aggagacagt ccaggccaag 420
tag 423

Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe
1 5 10 15
Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro
20 25 30
Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly
35 40 45
Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala
50 55 60
Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser
65 70 75 80
Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala
85 90 95
Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu
100 105 110
Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys
115 120 125
Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
130 135 140

FIG.10O

```
atgacagtgc ctcgaaagtc ggacctgttc caggaggacc tgtacccacc caccgcaggg   60
cccgaccctg ccctcacggc tgaggagtgg ctgggggggtc gggatgctgg gcccctcctc  120
atctccctca aggatggcta cgtaccccca aagagccggg agctgagggt caaccggggc  180
ctggacaccg ggcgcaggag ggcagcacca gaggccagtg gcactcccag ctcggatgcc  240
gtgtctcggc tggaggagga gatgcggaag ctccaggcca cggtgcagga gctccagaag  300
cgcttggaca ggctggagga gacagtccag gccaagtag                          339
```

```
Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro
 1               5                  10                  15
Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly
            20                  25                  30
Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val
        35                  40                  45
Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly
    50                  55                  60
Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala
65                  70                  75                  80
Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln
                85                  90                  95
Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
            100                 105                 110
```

FIG.10P

```
atgctgggcc cctcctcatc tccctcaagg atggctacgt acccccaaag agccgggagc   60
tga                                                                  63
```

```
Met Leu Gly Pro Ser Ser Ser Pro Ser Arg Met Ala Thr Tyr Pro Gln
 1               5                  10                  15
Arg Ala Gly Ser
            20
```

FIG.10Q

```
atggctacgt acccccaaag agccgggagc tga   33
```

```
Met Ala Thr Tyr Pro Gln Arg Ala Gly Ser
 1               5                  10
```

FIG.10R

```
atgccgtgtc tcggctggag gaggagatgc ggaagctcca ggccacggtg caggagctcc   60
agaagcgctt ggacaggctg gaggagacag tccaggccaa gtagagcccc gcagggcctc  120
cagcagggtc agccattcac acccatccac tcacctccca ttcccagcca catggcagag  180
aaaaaaatca taataaaatg gctttatttt ctggtaaaaa aaaaaaaaaa gggcggcc    238
```

```
Met Pro Cys Leu Gly Trp Arg Arg Arg Cys Gly Ser Ser Arg Pro Arg
 1               5                  10                  15
Cys Arg Ser Ser Arg Ser Ala Trp Thr Gly Trp Arg Arg Gln Ser Arg
            20                  25                  30
Pro Ser Arg Ala Pro Gln Gly Leu Gln Gln Gly Gln Pro Phe Thr Pro
        35                  40                  45
Ile His Ser Pro Pro Ile Pro Ser His Met Ala Glu Lys Lys Ile Ile
    50                  55                  60
Ile Lys Trp Leu Tyr Phe Leu Val Lys Lys Lys Lys Lys Gly Gly
65                  70                  75
```

FIG. 10S

```
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag   60
acagtccagg ccaagtag                                                  78
```

```
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
 1               5                  10                  15
Arg Leu Glu Glu Thr Val Gln Ala Lys
            20                  25
```

FIG. 10T

```
atggcagaga aaaaaatcat aataaaatgg ctttattttc tggtaaaaaa aaaaaaaaag   60
ggcggcc
```

```
Met Ala Glu Lys Lys Ile Ile Ile Lys Trp Leu Tyr Phe Leu Val Lys
 1               5                  10                  15
Lys Lys Lys Lys Gly Gly
            20
```

FIG. 10U

```
atggctttat tttctggtaa aaaaaaaaaa aaagggcggc c    41
```

```
Met Ala Leu Phe Ser Gly Lys Lys Lys Lys Lys Gly Arg
 1               5                  10
```

FIG. 10V

```
atgttcgcct gcgccaagct cgcctgcacc ccctctctga tccgagctgg atccagagtt   60
gcatacagac caatttctgc atcagtgtta tctcgaccag aggctagtag gactggagag  120
ggctctacgg tatttaatgg ggcccagaat ggtgtgtctc agctaatcca aagggagttt  180
cagaccagtg caatcagcag agacattgat actgctgcca aatttattgg tgcaggtgct  240
gcaacagtag gagtggctgg ttctggtgct ggtattggaa cagtctttgg cagccttatc  300
attggttatg ccagaaaccc ttcgctgaag cagcagctgt tctcatatgc tatcctggga  360
tttgccttgt ctgaagctat gggtctcttt tgtttgatgg ttgctttctt gattttgttt  420
gccatgtaa                                                          429
```

```
Met Phe Ala Cys Ala Lys Leu Ala Cys Thr Pro Ser Leu Ile Arg Ala
 1               5                  10                  15
Gly Ser Arg Val Ala Tyr Arg Pro Ile Ser Ala Ser Val Leu Ser Arg
            20                  25                  30
Pro Glu Ala Ser Arg Thr Gly Glu Gly Ser Thr Val Phe Asn Gly Ala
        35                  40                  45
Gln Asn Gly Val Ser Gln Leu Ile Gln Arg Glu Phe Gln Thr Ser Ala
    50                  55                  60
Ile Ser Arg Asp Ile Asp Thr Ala Ala Lys Phe Ile Gly Ala Gly Ala
65                  70                  75                  80
Ala Thr Val Gly Val Ala Gly Ser Gly Ala Gly Ile Gly Thr Val Phe
                85                  90                  95
Gly Ser Leu Ile Ile Gly Tyr Ala Arg Asn Pro Ser Leu Lys Gln Gln
            100                 105                 110
Leu Phe Ser Tyr Ala Ile Leu Gly Phe Ala Leu Ser Glu Ala Met Gly
        115                 120                 125
Leu Phe Cys Leu Met Val Ala Phe Leu Ile Leu Phe Ala Met
    130                 135                 140
```

FIG.11A

```
atggggccca gaatggtgtg tctcagctaa   30
```

```
Met Gly Pro Arg Met Val Cys Leu Ser
 1               5
```

FIG.11B

```
atggtgtgtc tcagctaa   18
```

```
Met Val Cys Leu Ser
 1               5
```

FIG.11C

EP 2U048 (1)



atgccagaaa cccttcgctg a 21

Met Pro Glu Thr Leu Arg
1 5

FIG.11D

atgctatcct gggatttgcc ttgtctgaag ctatgggtct cttttgtttg a 51

Met Leu Ser Trp Asp Leu Pro Cys Leu Lys Leu Trp Val Ser Phe Val
1 5 10 15

FIG.11E

atgggtctct tttgtttgat ggttgctttc ttgattttgt ttgccatgta a 51

Met Gly Leu Phe Cys Leu Met Val Ala Phe Leu Ile Leu Phe Ala Met
1 5 10 15

FIG.11F

atggttgctt tcttgatttt gtttgccatg taa 33

Met Val Ala Phe Leu Ile Leu Phe Ala Met
1 5 10

FIG.11G

atgttggcat tcatattaat tacggatgta attctgtgta tcttactgtg a 51

Met Leu Ala Phe Ile Leu Ile Thr Asp Val Ile Leu Cys Ile Leu Leu
1 5 10 15

FIG.11H

atgggaatgt acgttatttc caaagtcatt tcattaaaga tgaaaacttt aaaaaaaaaa 60
aaaaaagggc ggcc 74

Met Gly Met Tyr Val Ile Ser Lys Val Ile Ser Leu Lys Met Lys Thr
1 5 10 15
Leu Lys Lys Lys Lys Lys Gly Arg
20

FIG.11I

atgaagacag agccccaccc tcagatgcac atgagctggc gggattga 48

Met Lys Thr Glu Pro His Pro Gln Met His Met Ser Trp Arg Asp
1 5 10 15

FIG.12A

atgcacatga gctggcggga ttga 24

Met His Met Ser Trp Arg Asp
1 5

FIG.12B

atgagctggc gggattga 18

Met Ser Trp Arg Asp
1 5

FIG.12C

atgctgtctt cgtactggga aagggatttt cagccctcag aatcgctcca ccttgcagct 60
ctccccttct ctgtattcct agaaactgac acatgctga 99

Met Leu Ser Ser Tyr Trp Glu Arg Asp Phe Gln Pro Ser Glu Ser Leu
1 5 10 15
His Leu Ala Ala Leu Pro Phe Ser Val Phe Leu Glu Thr Asp Thr Cys
20 25 30

FIG.12D

atgctgaaca tcacagctta tttcctcatt tttataatgt cccttcacaa acccagtgtt 60
ttaggagcat ga 72

Met Leu Asn Ile Thr Ala Tyr Phe Leu Ile Phe Ile Met Ser Leu His
1 5 10 15
Lys Pro Ser Val Leu Gly Ala
20

FIG.12E

atgtcccttc acaaacccag tgttttagga gcatga 36

Met Ser Leu His Lys Pro Ser Val Leu Gly Ala
1 5 10

FIG.12F

atgagtgccg tgtgtgtgcg tcctgtcgga gccctgtctc ctctctctgt aataaactca 60
tttctagcag aaaaaaaaaa aaaaaaaaaa gggcggcc 98

Met Ser Ala Val Cys Val Arg Pro Val Gly Ala Leu Ser Pro Leu Ser
1 5 10 15
Val Ile Asn Ser Phe Leu Ala Glu Lys Lys Lys Lys Lys Lys Gly Arg
20 25 30

FIG.12G

```
atgcaagcat ccccgttcca gtga   24

Met Gln Ala Ser Pro Phe Gln
 1               5
```

FIG. 13A

```
atgcagctca aaacgcttag cctagccaca cccccacggg aaacagcagt gattaacctt   60
tag                                                                  63

Met Gln Leu Lys Thr Leu Ser Leu Ala Thr Pro Pro Arg Glu Thr Ala
 1               5                  10                  15
Val Ile Asn Leu
            20
```

FIG. 13B

```
atgcttagcc ctaaacctca acagttaaat caacaaaact gctcgccaga acactacgag   60
ccacagctta aaactcaaag gacctggcgg tgcttcatac ccctctag                108

Met Leu Ser Pro Lys Pro Gln Gln Leu Asn Gln Gln Asn Cys Ser Pro
 1               5                  10                  15
Glu His Tyr Glu Pro Gln Leu Lys Thr Gln Arg Thr Trp Arg Cys Phe
            20                  25                  30
Ile Pro Leu
        35
```

FIG. 13C

```
atgaaggcta caaagtaa   18

Met Lys Ala Thr Lys
 1               5
```

FIG. 13D

```
atggggtggc aagaaatggg ctacattttc taccccagaa aactacgata g   51

Met Gly Trp Gln Glu Met Gly Tyr Ile Phe Tyr Pro Arg Lys Leu Arg
 1               5                  10                  15
```

FIG. 13E

atgggctaca ttttctaccc cagaaaacta cgatag 36

Met Gly Tyr Ile Phe Tyr Pro Arg Lys Leu Arg
1 5 10

FIG.13F

atgaaactta agggtcgaag gtggatttag 30

Met Lys Leu Lys Gly Arg Arg Trp Ile
1 5

FIG.13G

atggtaagtg tactggaaag tgcacttgga cgaaaaaaaa aaaaaaaaaa aaaaaaaaaa 60
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 120
aaaaaaaaaa aaaaaaaaaa aaaagggcgg cc 152

Met Val Ser Val Leu Glu Ser Ala Leu Gly Arg Lys Lys Lys Lys Lys
1 5 10 15
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
20 25 30
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
35 40 45
Gly Arg
50

FIG.13H

SEQUENCE LISTING

<110> Shawn Barney
Mary Beth Thomas
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Donald C. Lo

<120> COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CONDITIONS, DISORDERS, OR DISEASES INVOLVING CELL DEATH

<130> 10001-0006-999

<160> 342

<170> FastSEQ for Windows Version 4.0

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agaaaatagt aaaaaaatat tttaatgata taaaatcctt ggctgctagc taggagtcgc     180
tctgtgctat agtagaaaaa tatggagact gggagctgtg tgatctattt tcaccagtaa     240
ctgggtgact ttaaaaggcc tgtaacttgt acttgtctac ttttatccag ttctacactg     300
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ttctgagggc tgcgaaggca ggatatgttc caggcctccc tctatggctt gtagatggcc     720
atcttcatgg tcacatggca ttctccctgt agctctctgt ttccagactt cccctttttg     780
taaggatatc agtgatatta gattagggtc ttccctaagg acctcatttg acctgcctgg     840
gctcaagcta ttctcccacc tctgcctccc taagagctgg gattacaggc atgagccatc     900
acacccgccc ctcattttaa tttgattacc tctgtaaata cctctgtctc caaatgagat     960
ttcatcctga gcagctgggg gttaggactt caatatatga atttgacagg gagggtagaa    1020
ggagagaaca gaattcaacc cacagcagca acaatctaat agcttcctgt gagcaagcaa    1080
agagaatgtt cattgtcagt ctcataggcg ccattcccta ttcatacgtt acttgtgctc    1140
tctcatattc cttgagtgtt ttaaattgta aacattcaag tacaaacaaa cttcgcttga    1200
ttaccagaga taaaaaagaa atgccttgta atttggtgtc atgtgaatgt tttaagtgga    1260
tacctgaaaa attgtactta agaatggcat aagagctttc tgattttcat tttacttcca    1320
ttaaagggga aaatatgcat agactgtcta tccattagcc agaacaatgg gacctctccc    1380
atcttaaaat aaaagccaaa ataatctggc caccaggaag aaagggtaga gcttgggaat    1440
gtcctcagga gattgtaaag atgcgtttcc ttgattcttt tgctcacact cttccctgtg    1500
actatttcct ccttcagggc tctatttctg ggttgggaga atgctgttcc agcaccaagc    1560
agtgtgggta tatatattca taccaaagag gcaatttgat tgtccttgga gttacaaaaa    1620
accaaatgtc aatgcctgat tagggaatac aacaataaaa gtaaaaataa tttaggagta    1680
tatatgcaga acatcagcct ttaaagtaat cttttattag gaaaatggca ttcacgattt    1740
gagaagatgg aaggtggtgg ggaacagaaa taaaaaaaaa aaaaaagggc ggcc          1794
```

<210> 2
<211> 39
<212> DNA
<213> Homo Sapiens

<400> 2
```
atggagactg ggagctgtgt gatctatttt caccagtaa                             39
```

<210> 3
<211> 12
<212> PRT
<213> Homo Sapiens

<400> 3
Met Glu Thr Gly Ser Cys Val Ile Tyr Phe His Gln
1 5 10

<210> 4
<211> 45
<212> DNA
<213> Homo Sapiens

<400> 4
atgattctca acatcttttt ctggtatgta agactttcct catga 45

<210> 5
<211> 14
<212> PRT
<213> Homo Sapiens

<400> 5
Met Ile Leu Asn Ile Phe Phe Trp Tyr Val Arg Leu Ser Ser
1 5 10

<210> 6
<211> 51
<212> DNA
<213> Homo Sapiens

<400> 6
atgaaattca gaacattgcc atttaaggaa tggcaaagat tttttcccta a 51

<210> 7
<211> 16
<212> PRT
<213> Homo Sapiens

<400> 7
Met Lys Phe Arg Thr Leu Pro Phe Lys Glu Trp Gln Arg Phe Phe Pro
1 5 10 15

<210> 8
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 8
atggcaaaga tttttttccct aaagttaaaa gatcaaatat ga 42

<210> 9
<211> 13
<212> PRT
<213> Homo Sapiens

<400> 9
Met Ala Lys Ile Phe Ser Leu Lys Leu Lys Asp Gln Ile
1 5 10

<210> 10
<211> 15
<212> DNA
<213> Homo Sapiens

<400> 10
atgaaattaa tataa 15

<210> 11
<211> 4
<212> PRT
<213> Homo Sapiens

<400> 11
Met Lys Leu Ile
1

<210> 12
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 12
atgtacagtt ga 12

<210> 13
<211> 3
<212> PRT
<213> Homo Sapiens

<400> 13
Met Tyr Ser
1

<210> 14
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 14
atgtcaaaaa ttgactttca tttatag 27

<210> 15
<211> 8
<212> PRT
<213> Homo Sapiens

<400> 15
Met Ser Lys Ile Asp Phe His Leu
1 5

<210> 16
<211> 51
<212> DNA
<213> Homo Sapiens

<400> 16
atgtattatc tcacagttct gcagtctaga agtctggaat caaggtgtta g 51

<210> 17
<211> 16
<212> PRT
<213> Homo Sapiens

<400> 17
Met Tyr Tyr Leu Thr Val Leu Gln Ser Arg Ser Leu Glu Ser Arg Cys
1 5 10 15

<210> 18
<211> 30

<212> DNA
<213> Homo Sapiens

<400> 18
atgttccagg cctccctcta tggcttgtag 30

<210> 19
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 19
Met Phe Gln Ala Ser Leu Tyr Gly Leu
1 5

<210> 20
<211> 221
<212> DNA
<213> Homo Sapiens

<400> 20
atggcttgta gatggccatc ttcatggtca catggcattc tccctgtagc tctctgtttc 60
cagacttccc ctttttgtaa ggatatcagt gatattagat tagggtcttc cctaaggacc 120
catttgacct gcctgggctc aagctattct cccacctctg cctccctaag agctgggatt 180
acaggcatga gccatcacac ccgcccctca ttttaatttg a 221

<210> 21
<211> 73
<212> PRT
<213> Homo Sapiens

<400> 21
Met Ala Cys Arg Trp Pro Ser Ser Trp Ser His Gly Ile Leu Pro Val
1 5 10 15
Ala Leu Cys Phe Gln Thr Ser Pro Phe Cys Lys Asp Ile Ser Asp Ile
20 25 30
Arg Leu Gly Ser Ser Leu Arg Thr Ser Phe Asp Leu Pro Gly Leu Lys
35 40 45
Leu Phe Ser His Leu Cys Leu Pro Lys Ser Trp Asp Tyr Arg His Glu
50 55 60
Pro Ser His Pro Pro Leu Ile Leu Ile
65 70

<210> 22
<211> 69
<212> DNA
<213> Homo Sapiens

<400> 22
atggccatct tcatggtcac atggcattct ccctgtagct ctctgtttcc agacttcccc 60
tttttgtaa 69

<210> 23
<211> 22
<212> PRT
<213> Homo Sapiens

<400> 23
Met Ala Ile Phe Met Val Thr Trp His Ser Pro Cys Ser Ser Leu Phe
1 5 10 15
Pro Asp Phe Pro Phe Leu
20

<210> 24
<211> 57

<212> DNA
<213> Homo Sapiens

<400> 24
atggtcacat ggcattctcc ctgtagctct ctgtttccag acttcccctt tttgtaa 57

<210> 25
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<212> PRT
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<400> 25
Met Val Thr Trp His Ser Pro Cys Ser Ser Leu Phe Pro Asp Phe Pro
1 5 10 15
Phe Leu

<210> 26
<211> 18
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<400> 26
atggcattct ccctgtag 18

<210> 27
<211> 5
<212> PRT
<213> Homo Sapiens

<400> 27
Met Ala Phe Ser Leu
1 5

<210> 28
<211> 30
<212> DNA
<213> Homo Sapiens

<400> 28
atgagccatc acacccgccc ctcattttaa 30

<210> 29
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 29
Met Ser His His Thr Arg Pro Ser Phe
1 5

<210> 30
<211> 339
<212> DNA
<213> Homo Sapiens

<400> 30
atgagatttc atcctgagca gctgggggtt aggacttcaa tatatgaatt tgacagggag 60
ggtagaagga gagaacagaa ttcaacccac agcagcaaca atctaatagc ttcctgtgag 120
caagcaaaga gaatgttcat tgtcagtctc ataggcgcca ttccctattc atacgttact 180
tgtgctctct catattcctt gagtgtttta aattgtaaac attcaagtac aaacaaactt 240
cgcttgatta ccagagataa aaaagaaatg ccttgtaatt tggtgtcatg tgaatgtttt 300
aagtggatac ctgaaaaatt gtacttaaga atggcataa 339

<210> 31

<211> 112
<212> PRT
<213> Homo Sapiens

<400> 31
Met Arg Phe His Pro Glu Gln Leu Gly Val Arg Thr Ser Ile Tyr Glu
1 5 10 15
Phe Asp Arg Glu Gly Arg Arg Arg Glu Gln Asn Ser Thr His Ser Ser
20 25 30
Asn Asn Leu Ile Ala Ser Cys Glu Gln Ala Lys Arg Met Phe Ile Val
35 40 45
Ser Leu Ile Gly Ala Ile Pro Tyr Ser Tyr Val Thr Cys Ala Leu Ser
50 55 60
Tyr Ser Leu Ser Val Leu Asn Cys Lys His Ser Ser Thr Asn Lys Leu
65 70 75 80
Arg Leu Ile Thr Arg Asp Lys Lys Glu Met Pro Cys Asn Leu Val Ser
85 90 95
Cys Glu Cys Phe Lys Trp Ile Pro Glu Lys Leu Tyr Leu Arg Met Ala
100 105 110

<210> 32
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 32
atgaatttga cagggagggt agaaggagag aacagaattc aacccacagc agcaacaatc 60
taa 63

<210> 33
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 33
Met Asn Leu Thr Gly Arg Val Glu Gly Glu Asn Arg Ile Gln Pro Thr
1 5 10 15
Ala Ala Thr Ile
20

<210> 34
<211> 207
<212> DNA
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agagataaaa aagaaatgcc ttgtaatttg gtgtcatgtg aatgttttaa gtggatacct 180
gaaaaattgt acttaagaat ggcataa 207

<210> 35
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<212> PRT
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<400> 35
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20 25 30
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35 40 45
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50 55 60

Leu Arg Met Ala
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agaatggcat aa 72

<210> 37
<211> 23
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<400> 37
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Lys Leu Tyr Leu Arg Met Ala
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<210> 39
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<212> PRT
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<400> 39
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<210> 40
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<212> DNA
<213> Homo Sapiens

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<210> 41
<211> 6
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<213> Homo Sapiens

<400> 41
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<210> 42
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<212> DNA
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<400> 42
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<210> 43
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<212> PRT
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<400> 43
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<210> 44
<211> 183
<212> DNA
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<210> 45
<211> 60
<212> PRT
<213> Homo Sapiens

<400> 45
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20 25 30
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35 40 45
Leu Gly Val Thr Lys Asn Gln Met Ser Met Pro Asp
50 55 60

<210> 46
<211> 99
<212> DNA
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tgtccttgga gttacaaaaa accaaatgtc aatgcctga 99

<210> 47
<211> 32
<212> PRT
<213> Homo Sapiens

<400> 47
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20 25 30

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<400> 49
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<212> DNA
<213> Homo Sapiens

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<210> 53
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<400> 53
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<210> 54
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<210> 57
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<210> 58
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cacatagact tggcaagagt aaggagggaa ctccatagag acattttacc tatctcaggg 180
gagcagccac aaagaagcaa gtcttgtaaa aggtcttttg caaaggagag tgaacccagc 240
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ctactgaaca aaaaaagaaa aagggcggcc 810

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<210> 60
<211> 4
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<213> Homo Sapiens

<400> 60
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<210> 61
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 61
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<210> 62
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<400> 62
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<210> 63

<211> 12
<212> DNA
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<400> 63
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<210> 64
<211> 3
<212> PRT
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<400> 64
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<210> 65
<211> 51
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<400> 66
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<210> 67
<211> 33
<212> DNA
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<400> 67
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<210> 68
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<400> 68
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1 5 10

<210> 69
<211> 15
<212> DNA
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<400> 69
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<400> 70
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<210> 71
<211> 135
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aggtgggtgt ggtga 135

<210> 72
<211> 44
<212> PRT
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<400> 72
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20 25 30
Glu Asp Ser Ser Ser Thr Cys Pro Arg Trp Val Trp
35 40

<210> 73
<211> 57
<212> DNA
<213> Homo Sapiens

<400> 73
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<210> 74
<211> 18
<212> PRT
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<400> 74
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1 5 10 15
Val Trp

<210> 75
<211> 96
<212> DNA
<213> Homo Sapiens

<400> 75
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tcctttatta gcccacattc tgatgttccc tggtga 96

<210> 76
<211> 31
<212> PRT
<213> Homo Sapiens

<400> 76
Met Met Arg Lys Asp Lys Arg Leu Ala Arg Thr Leu Lys Arg Ser Glu
1 5 10 15
His His Thr Asp Ser Phe Ile Ser Pro His Ser Asp Val Pro Trp
20 25 30

<210> 77

<211> 93
<212> DNA
<213> Homo Sapiens

<400> 77
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tttattagcc cacattctga tgttccctgg tga 93

<210> 78
<211> 30
<212> PRT
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<400> 78
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1 5 10 15
His Thr Asp Ser Phe Ile Ser Pro His Ser Asp Val Pro Trp
20 25 30

<210> 79
<211> 51
<212> DNA
<213> Homo Sapiens

<400> 79
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<210> 80
<211> 16
<212> PRT
<213> Homo Sapiens

<400> 80
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<210> 81
<211> 57
<212> DNA
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<400> 81
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<210> 82
<211> 19
<212> PRT
<213> Homo Sapiens

<400> 82
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Arg Ala Ala

<210> 83
<211> 920
<212> DNA
<213> Homo Sapiens

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gcctgtttac caaaaacatc acctctagca tcaccagtat tagaggcacc gcctgcccag 180
tgacacatgt ttaacggccg cggtacccta accgtgcaaa ggtagcataa tcacttgttc 240

cttaattagg gacctgtatg aatggctcca cgagggttca gctgtctctt acttttaacc 300
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caacggaaca agttacccta gggataacag cgcaatccta ttctagagtc catatcaaca 600
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<400> 84
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<210> 85
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<400> 85
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<210> 86
<211> 39
<212> DNA
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<400> 86
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<210> 87
<211> 12
<212> PRT
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<400> 87
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<210> 88
<211> 48
<212> DNA
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<400> 88
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<210> 89
<211> 15
<212> PRT
<213> Homo Sapiens

<400> 89
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1 5 10 15

<210> 90

<211> 75
<212> DNA
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<400> 90
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<210> 91
<211> 24
<212> PRT
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<400> 91
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<210> 92
<211> 99
<212> DNA
<213> Homo Sapiens

<400> 92
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aacaaaccca caggtcctaa actaccaaac ctgcattaa 99

<210> 93
<211> 32
<212> PRT
<213> Homo Sapiens

<400> 93
Met Thr Gln Gln Asp Glu Lys Thr Leu Trp Ser Phe Asn Leu Leu Met
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<211> 12
<212> DNA
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<400> 94
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<210> 95
<211> 3
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<400> 95
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<210> 96
<211> 54
<212> DNA
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<400> 96
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<210> 97

<211> 17
<212> PRT
<213> Homo Sapiens

<400> 97
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1 5 10 15
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<210> 98
<211> 45
<212> DNA
<213> Homo Sapiens

<400> 98
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<210> 99
<211> 14
<212> PRT
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<400> 99
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<210> 100
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 100
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taa 63

<210> 101
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 101
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1 5 10 15
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<210> 102
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 102
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<210> 103
<211> 13
<212> PRT
<213> Homo Sapiens

<400> 103
Met Val Gln Pro Leu Leu Lys Val Arg Leu Phe Asn Asp
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<210> 104

<211> 116
<212> DNA
<213> Homo Sapiens

<400> 104
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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagg gcggcc 116

<210> 105
<211> 38
<212> PRT
<213> Homo Sapiens

<400> 105
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Lys Lys Lys Lys Gly Arg
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<212> DNA
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aaccttagtt ctaaggattt aacatcctgt aagtgaagtt taacataaca gtattccata 2040
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ttaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaagggc ggcc 2144

<210> 107

<211> 444
<212> DNA
<213> Homo Sapiens

<400> 107
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gaagacatga cacttacaag atggacaggg atgataattg ggcctccaag aacaatttat 180
gaaaaccgaa tatacagcct taaaatagaa tgtggaccta aatacccaga agcacccccc 240
tttgtaagat ttgtaacaaa aattaatatg aatggagtaa atagttctaa tggagtggtg 300
gacccaagag ccatatcagt gctagcaaaa tggcagaatt catatagcat caaagttgtc 360
ctgcaagagc ttcggcgcct aatgatgtct aaagaaaata tgaaactccc tcagccgccc 420
gaaggacagt gttacagcaa ttaa 444

<210> 108
<211> 147
<212> PRT
<213> Homo Sapiens

<400> 108
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Val Ser Trp Gly Leu Glu Asp Asp Glu Asp Met Thr Leu Thr Arg Trp
35 40 45
Thr Gly Met Ile Ile Gly Pro Pro Arg Thr Ile Tyr Glu Asn Arg Ile
50 55 60
Tyr Ser Leu Lys Ile Glu Cys Gly Pro Lys Tyr Pro Glu Ala Pro Pro
65 70 75 80
Phe Val Arg Phe Val Thr Lys Ile Asn Met Asn Gly Val Asn Ser Ser
85 90 95
Asn Gly Val Val Asp Pro Arg Ala Ile Ser Val Leu Ala Lys Trp Gln
100 105 110
Asn Ser Tyr Ser Ile Lys Val Val Leu Gln Glu Leu Arg Arg Leu Met
115 120 125
Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys
130 135 140
Tyr Ser Asn
145

<210> 109
<211> 24
<212> DNA
<213> Homo Sapiens

<400> 109
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<210> 110
<211> 7
<212> PRT
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<400> 110
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<210> 111
<211> 15
<212> DNA
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<400> 111
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<210> 112
<211> 4
<212> PRT
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<400> 112
Met Thr Lys Thr
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<210> 113
<211> 318
<212> DNA
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agatttgtaa caaaaattaa tatgaatgga gtaaatagtt ctaatggagt ggtggaccca 180
agagccatat cagtgctagc aaaatggcag aattcatata gcatcaaagt tgtcctgcaa 240
gagcttcggc gcctaatgat gtctaaagaa aatatgaaac tccctcagcc gcccgaagga 300
cagtgttaca gcaattaa 318

<210> 114
<211> 105
<212> PRT
<213> Homo Sapiens

<400> 114
Met Thr Leu Thr Arg Trp Thr Gly Met Ile Ile Gly Pro Pro Arg Thr
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Ile Tyr Glu Asn Arg Ile Tyr Ser Leu Lys Ile Glu Cys Gly Pro Lys
            20                  25                  30
Tyr Pro Glu Ala Pro Pro Phe Val Arg Phe Val Thr Lys Ile Asn Met
        35                  40                  45
Asn Gly Val Asn Ser Ser Asn Gly Val Val Asp Pro Arg Ala Ile Ser
    50                  55                  60
Val Leu Ala Lys Trp Gln Asn Ser Tyr Ser Ile Lys Val Val Leu Gln
65                  70                  75                  80
Glu Leu Arg Arg Leu Met Met Ser Lys Glu Asn Met Lys Leu Pro Gln
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Pro Pro Glu Gly Gln Cys Tyr Ser Asn
            100                 105

<210> 115
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 115
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<210> 116
<211> 13
<212> PRT
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<400> 116
Met Asp Arg Asp Asp Asn Trp Ala Ser Lys Asn Asn Leu
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<210> 117
<211> 294
<212> DNA
<213> Homo Sapiens

<400> 117
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tgtggaccta aatacccaga agcacccccc tttgtaagat ttgtaacaaa aattaatatg 120
aatggagtaa atagttctaa tggagtggtg gacccaagag ccatatcagt gctagcaaaa 180
tggcagaatt catatagcat caaagttgtc ctgcaagagc ttcggcgcct aatgatgtct 240
aaagaaaata tgaaactccc tcagccgccc gaaggacagt gttacagcaa ttaa 294

<210> 118
<211> 97
<212> PRT
<213> Homo Sapiens

<400> 118
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1 5 10 15
Leu Lys Ile Glu Cys Gly Pro Lys Tyr Pro Glu Ala Pro Pro Phe Val
20 25 30
Arg Phe Val Thr Lys Ile Asn Met Asn Gly Val Asn Ser Ser Asn Gly
35 40 45
Val Val Asp Pro Arg Ala Ile Ser Val Leu Ala Lys Trp Gln Asn Ser
50 55 60
Tyr Ser Ile Lys Val Val Leu Gln Glu Leu Arg Arg Leu Met Met Ser
65 70 75 80
Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys Tyr Ser
85 90 95
Asn

<210> 119
<211> 30
<212> DNA
<213> Homo Sapiens

<400> 119
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<210> 120
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 120
Met Lys Thr Glu Tyr Thr Ala Leu Lys
1 5

<210> 121
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 121
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<210> 122
<211> 3
<212> PRT
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<400> 122
Met Trp Thr
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<210> 123
<211> 177
<212> DNA

<213> Homo Sapiens

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tctaaagaaa atatgaaact ccctcagccg cccgaaggac agtgttacag caattaa 177

<210> 124
<211> 58
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<400> 124
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1 5 10 15
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20 25 30
Gln Glu Leu Arg Arg Leu Met Met Ser Lys Glu Asn Met Lys Leu Pro
35 40 45
Gln Pro Pro Glu Gly Gln Cys Tyr Ser Asn
50 55

<210> 125
<211> 36
<212> DNA
<213> Homo Sapiens

<400> 125
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<210> 126
<211> 11
<212> PRT
<213> Homo Sapiens

<400> 126
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1 5 10

<210> 127
<211> 18
<212> DNA
<213> Homo Sapiens

<400> 127
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<210> 128
<211> 5
<212> PRT
<213> Homo Sapiens

<400> 128
Met Ala Glu Phe Ile
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<210> 129
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 129
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taa 63

<210> 130
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 130
Met Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln
1 5 10 15
Cys Tyr Ser Asn
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<210> 131
<211> 60
<212> DNA
<213> Homo Sapiens

<400> 131
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<210> 132
<211> 19
<212> PRT
<213> Homo Sapiens

<400> 132
Met Ser Lys Glu Asn Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys
1 5 10 15
Tyr Ser Asn

<210> 133
<211> 45
<212> DNA
<213> Homo Sapiens

<400> 133
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<210> 134
<211> 14
<212> PRT
<213> Homo Sapiens

<400> 134
Met Lys Leu Pro Gln Pro Pro Glu Gly Gln Cys Tyr Ser Asn
1 5 10

<210> 135
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 135
atgatactaa ttttttcgtc catttga 27

<210> 136
<211> 8
<212> PRT
<213> Homo Sapiens

<400> 136
Met Ile Leu Ile Phe Ser Ser Ile
1 5

<210> 137

<211> 72
<212> DNA
<213> Homo Sapiens

<400> 137
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catagattgt aa 72

<210> 138
<211> 23
<212> PRT
<213> Homo Sapiens

<400> 138
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1 5 10 15
Pro Trp Pro Lys His Arg Leu
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<210> 139
<211> 57
<212> DNA
<213> Homo Sapiens

<400> 139
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<210> 140
<211> 18
<212> PRT
<213> Homo Sapiens

<400> 140
Met Thr Leu Pro Cys Phe Gly Gln Lys Pro Ser Gly Lys Val Gly Lys
1 5 10 15
Ser Leu

<210> 141
<211> 48
<212> DNA
<213> Homo Sapiens

<400> 141
atgaatattg ggtcctcagc cctgccaccc tctgctgtca tcagctga 48

<210> 142
<211> 15
<212> PRT
<213> Homo Sapiens

<400> 142
Met Asn Ile Gly Ser Ser Ala Leu Pro Pro Ser Ala Val Ile Ser
1 5 10 15

<210> 143
<211> 15
<212> DNA
<213> Homo Sapiens

<400> 143
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<210> 144
<211> 4

<212> PRT
<213> Homo Sapiens

<400> 144
Met His Cys Phe
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<210> 145
<211> 78
<212> DNA
<213> Homo Sapiens

<400> 145
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tttgctttta aaaattga 78

<210> 146
<211> 25
<212> PRT
<213> Homo Sapiens

<400> 146
Met Lys Ile Leu Val Ser Thr His Asp Pro Ser Glu Leu Phe Tyr Phe
1 5 10 15
Pro Val Thr Ala Phe Ala Phe Lys Asn
20 25

<210> 147
<211> 33
<212> DNA
<213> Homo Sapiens

<400> 147
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<210> 148
<211> 10
<212> PRT
<213> Homo Sapiens

<400> 148
Met Ile Pro Leu Asn Cys Phe Thr Phe Leu
1 5 10

<210> 149
<211> 156
<212> DNA
<213> Homo Sapiens

<400> 149
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cccggtgtgc acagcagatc cccgaaattg gtgggcttga cctcctggca aattgctgcg 120
tctttccact tgctgttcag gaccactaaa tgctga 156

<210> 150
<211> 51
<212> PRT
<213> Homo Sapiens

<400> 150
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1 5 10 15
Ile Lys Lys Glu Pro Gly Val His Ser Arg Ser Pro Lys Leu Val Gly
20 25 30
Leu Thr Ser Trp Gln Ile Ala Ala Ser Phe His Leu Leu Phe Arg Thr

35 40 45
Thr Lys Cys
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<210> 151
<211> 30
<212> DNA
<213> Homo Sapiens

<400> 151
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<210> 152
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 152
Met Leu Lys Cys Gly Cys Ile Pro Lys
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<210> 153
<211> 69
<212> DNA
<213> Homo Sapiens

<400> 153
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ttaatttag 69

<210> 154
<211> 22
<212> PRT
<213> Homo Sapiens

<400> 154
Met Trp Met His Thr Glu Ile Lys Ala Ile His Cys Val Leu Lys Val
1 5 10 15
Phe Phe Phe Phe Leu Ile
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<210> 155
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 155
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<210> 156
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 156
Met His Thr Glu Ile Lys Ala Ile His Cys Val Leu Lys Val Phe Phe
1 5 10 15
Phe Phe Leu Ile
20

<210> 157
<211> 66
<212> DNA
<213> Homo Sapiens

<400> 157
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aagtga 66

<210> 158
<211> 21
<212> PRT
<213> Homo Sapiens

<400> 158
Met Phe Pro Leu Ile Phe Phe Trp Gly Glu Asn Leu Ser Ser Lys Asp
1 5 10 15
Leu Thr Ser Cys Lys
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<210> 159
<211> 1293
<212> DNA
<213> Homo Sapiens

<400> 159
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agccgagatc acaccactgc actccagcct gggtgacaag aacgaaactc catctcaaaa 120
aataaaataa aatatatact atcttgctcc tcagaaccag tggggaagaa gagggaaggc 180
aaagaaagaa actgagcata gtaaacacag catttttttg taggctctta tttaaaatgt 240
gtgtgtgtgt gtgtatgtgt gtgtttctga gtaagtattg actgggaaaa agagagaagt 300
caatcaaaag tatactgtgc aattgagaga ggctggccca agatttaaaa cttcctgtgg 360
gtaatctaac tgtgagtaga taggaatcgg ccatatgacg aaatgagatc aataggaaat 420
gtgctttttg aggaaatttt attttagtac caaatgttgc cagtgacaat cttcagttaa 480
gaagtaagtt atttgaccta aaattcttat ctctgccact ttggtttaaa aacaaaaacc 540
cttatataca tggaatagtt atattttaat taagcattta ttttagttgt tttcatccat 600
tcaagcaaaa tgaataagca gcatttttca ttgcacttaa aaatgtaaaa tacctgcatg 660
ccactaatct gtaacatttt accagttcag atgcctgtaa tgtgtgactt tatgtgtgtc 720
tgtgttgttt tgaagagaat aaaggaaata atactttgca aactgtttaa acaagtgttt 780
aaacttctat tggcaacatt tattgggcta agcagttatt gaaaactccg catagtttta 840
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aaatgtaaac tgttttaatt aattaagatc tttctgcttt cgaaggtata atgtatctat 1140
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<210> 160
<211> 45
<212> DNA
<213> Homo Sapiens

<400> 160
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<210> 161
<211> 14
<212> PRT
<213> Homo Sapiens

<400> 161
Met Cys Val Cys Val Cys Met Cys Val Phe Leu Ser Lys Tyr
1 5 10

<210> 162
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 162
atgtgtgtgt ttctgagtaa gtattga 27

<210> 163
<211> 8
<212> PRT
<213> Homo Sapiens

<400> 163
Met Cys Val Phe Leu Ser Lys Tyr
1 5

<210> 164
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 164
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<210> 165
<211> 3
<212> PRT
<213> Homo Sapiens

<400> 165
Met Thr Lys
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<210> 166
<211> 45
<212> DNA
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<400> 166
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<210> 167
<211> 14
<212> PRT
<213> Homo Sapiens

<400> 167
Met Arg Ser Ile Gly Asn Val Leu Phe Glu Glu Ile Leu Phe
1 5 10

<210> 168
<211> 48
<212> DNA
<213> Homo Sapiens

<400> 168
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<210> 169
<211> 15
<212> PRT
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<400> 169
Met Cys Phe Leu Arg Lys Phe Tyr Phe Ser Thr Lys Cys Cys Gln
1 5 10 15

<210> 170
<211> 27

<212> DNA
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<400> 170
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<210> 171
<211> 8
<212> PRT
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<400> 171
Met Leu Pro Val Thr Ile Phe Ser
1 5

<210> 172
<211> 39
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<400> 172
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<210> 173
<211> 12
<212> PRT
<213> Homo Sapiens

<400> 173
Met Asn Lys Gln His Phe Ser Leu His Leu Lys Met
1 5 10

<210> 174
<211> 165
<212> DNA
<213> Homo Sapiens

<400> 174
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gtctgtgttg ttttgaagag aataaaggaa ataatacttt gcaaactgtt taaacaagtg 120
tttaaacttc tattggcaac atttattggg ctaagcagtt attga 165

<210> 175
<211> 54
<212> PRT
<213> Homo Sapiens

<400> 175
Met Pro Leu Ile Cys Asn Ile Leu Pro Val Gln Met Pro Val Met Cys
1 5 10 15
Asp Phe Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu Ile Ile
20 25 30
Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala Thr Phe
35 40 45
Ile Gly Leu Ser Ser Tyr
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<210> 176
<211> 132
<212> DNA
<213> Homo Sapiens

<400> 176
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atactttgca aactgtttaa acaagtgttt aaacttctat tggcaacatt tattgggcta 120

agcagttatt ga 132

<210> 177
<211> 43
<212> PRT
<213> Homo Sapiens

<400> 177
Met Pro Val Met Cys Asp Phe Met Cys Val Cys Val Val Leu Lys Arg
1 5 10 15
Ile Lys Glu Ile Ile Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu
20 25 30
Leu Leu Ala Thr Phe Ile Gly Leu Ser Ser Tyr
35 40

<210> 178
<211> 123
<212> DNA
<213> Homo Sapiens

<400> 178
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aaactgttta aacaagtgtt taaacttcta ttggcaacat ttattgggct aagcagttat 120
tga 123

<210> 179
<211> 40
<212> PRT
<213> Homo Sapiens

<400> 179
Met Cys Asp Phe Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu
1 5 10 15
Ile Ile Leu Cys Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala
20 25 30
Thr Phe Ile Gly Leu Ser Ser Tyr
35 40

<210> 180
<211> 111
<212> DNA
<213> Homo Sapiens

<400> 180
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caagtgttta aacttctatt ggcaacattt attgggctaa gcagttattg a 111

<210> 181
<211> 36
<212> PRT
<213> Homo Sapiens

<400> 181
Met Cys Val Cys Val Val Leu Lys Arg Ile Lys Glu Ile Ile Leu Cys
1 5 10 15
Lys Leu Phe Lys Gln Val Phe Lys Leu Leu Leu Ala Thr Phe Ile Gly
20 25 30
Leu Ser Ser Tyr
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<210> 182
<211> 57
<212> DNA
<213> Homo Sapiens

<400> 182
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<210> 183
<211> 18
<212> PRT
<213> Homo Sapiens

<400> 183
Met Cys Asp Glu Ala Lys Cys Ile Lys Tyr Glu Ile Leu Tyr Phe Tyr
1 5 10 15
Pro Gly

<210> 184
<211> 18
<212> DNA
<213> Homo Sapiens

<400> 184
atgaagcaaa atgtataa 18

<210> 185
<211> 5
<212> PRT
<213> Homo Sapiens

<400> 185
Met Lys Gln Asn Val
1 5

<210> 186
<211> 15
<212> DNA
<213> Homo Sapiens

<400> 186
atgtataaag tatga 15

<210> 187
<211> 4
<212> PRT
<213> Homo Sapiens

<400> 187
Met Tyr Lys Val
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<210> 188
<211> 99
<212> DNA
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<400> 188
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gcaattttta atcctttgaa attagccagc cagacctaa 99

<210> 189
<211> 32
<212> PRT
<213> Homo Sapiens

<400> 189
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1 5 10 15

Ala Gln Ile Gly Ala Ile Phe Asn Pro Leu Lys Leu Ala Ser Gln Thr
20 25 30

<210> 190
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 190
atgctaaggt aa 12

<210> 191
<211> 3
<212> PRT
<213> Homo Sapiens

<400> 191
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<210> 192
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 192
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taa 63

<210> 193
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 193
Met Tyr Leu Phe Leu Ser Gly Met Ile Phe Pro Asn Glu Asn Val Lys
1 5 10 15
Asn Ile Gly Lys
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<210> 194
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 194
atgatatttc caaatgaaaa tgtaaagaac attgggaaat aa 42

<210> 195
<211> 13
<212> PRT
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<400> 195
Met Ile Phe Pro Asn Glu Asn Val Lys Asn Ile Gly Lys
1 5 10

<210> 196
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 196
atgaaaatgt aa 12

<210> 197
<211> 3
<212> PRT
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<400> 197
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<210> 198
<211> 1466
<212> DNA
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<400> 198
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ttttgggtgt tagactgaaa tattttaaga gtatttaacc tttccagtat tctgtttcac     180
gcttagatgg aaatgtatct tatgaataga gacatattaa aataatgttt acatcttaga     240
aaaaacatag atagtgctag taatattact tataactgta atatatagat tcagaaatac     300
attttcatta tccaaaatca gcttcaacaa atggtttctg gagacaaata atttgttttc     360
attatcattg tataatcagg ttaatgattt atttttgac taaatgtgca atttcttatc-     420
actagataac tttcagtatc agtggtggtt acttattact taaatcagag gaaggatttt     480
ataaagatta ataaatttaa ttttaccaat aaatattccc ataatttaga aaaggatgtc     540
gacttgctaa tttcagaaat aattattcat ttttaaaaag cccctttttaa agcatctact     600
tgaagattgg tataattttc ataaaatgtc ttttttttta gtgtcccaaa gatatcttag     660
ataaactatt ttgaagttca gatttcagat gaggcaacat tttcttgaga taattaccca     720
agtttcatcc atgttgaatg gtacaaaata tttctgtgaa actaacagga agatattttc     780
agataactag gataacttgt tgctttgtta cccagcctaa ttgaagagtg gcagagacta     840
ctacaaaaag caacctttttc attttcacta agagtttaaa agctattgta ttattaaaaa     900
gtctttacaa tgcttgtttc aaagaaccaa cagaaaaaaa agctaagaaa actgagaact     960
aacattaaaa aaattaaatt tagaataaga atgatttctt taatttgtcc tttttttctt    1020
tggtctaaaa cattattaaa tttttgtaaa tattttgatt taatgtgtct tagatcctca    1080
ttattttaat acaggaaaag aaaagattta gtaatttctt accatgctaa tatgtaaagt    1140
tcatgccatc caggcattta agagcgatcc tcatcccttc agcaatatgt atttgagttc    1200
acactatttc tgttttacag cagttttgaa aaacacatac tatgccacca attgtcatat    1260
tatttttaga tgatgtaaca tagccatcaa aattaatatt atgtaatgcc taatacttag    1320
tatgtaaatg tcacgagatc atttttacat taaacgtgaa aaaaaatcaa aaaaaaaaaa    1380
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aaaaaaaaaa aaaaaaaagg gcggcc                                         1466
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<210> 199
<211> 129
<212> DNA
<213> Homo Sapiens

<400> 199
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<210> 200
<211> 42
<212> PRT
<213> Homo Sapiens

<400> 200
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Asp Ile Leu Trp Ile Pro Pro Leu His Trp Gln Asn Thr Met Leu Leu
            20                  25                  30
Gly Val Arg Leu Lys Tyr Phe Lys Ser Ile
        35                  40
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<210> 201

<211> 69
<212> DNA
<213> Homo Sapiens

<400> 201
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ttagactga 69

<210> 202
<211> 22
<212> PRT
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<400> 202
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1 5 10 15
Cys Phe Trp Val Leu Asp
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<210> 203
<211> 42
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<400> 203
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<210> 204
<211> 13
<212> PRT
<213> Homo Sapiens

<400> 204
Met Leu Leu Gly Val Arg Leu Lys Tyr Phe Lys Ser Ile
1 5 10

<210> 205
<211> 39
<212> DNA
<213> Homo Sapiens

<400> 205
atggaaatgt atcttatgaa tagagacata ttaaaataa 39

<210> 206
<211> 12
<212> PRT
<213> Homo Sapiens

<400> 206
Met Glu Met Tyr Leu Met Asn Arg Asp Ile Leu Lys
1 5 10

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<212> DNA
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<210> 208
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<400> 208
Met Tyr Leu Met Asn Arg Asp Ile Leu Lys
1 5 10

<210> 209
<211> 24
<212> DNA
<213> Homo Sapiens

<400> 209
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<210> 210
<211> 7
<212> PRT
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<400> 210
Met Asn Arg Asp Ile Leu Lys
1 5

<210> 211
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<400> 211
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<210> 212
<211> 4
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<213> Homo Sapiens

<400> 212
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<212> DNA
<213> Homo Sapiens

<400> 213
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<210> 214
<211> 6
<212> PRT
<213> Homo Sapiens

<400> 214
Met Val Ser Gly Asp Lys
1 5

<210> 215
<211> 42
<212> DNA
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<400> 215
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<210> 216
<211> 13

<212> PRT
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<400> 216
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 1               5                   10

<210> 217
<211> 60
<212> DNA
<213> Homo Sapiens

<400> 217
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<210> 218
<211> 19
<212> PRT
<213> Homo Sapiens

<400> 218
Met Cys Asn Phe Leu Ser Leu Asp Asn Phe Gln Tyr Gln Trp Trp Leu
 1               5                   10                  15
Leu Ile Thr

<210> 219
<211> 15
<212> DNA
<213> Homo Sapiens

<400> 219
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<210> 220
<211> 4
<212> PRT
<213> Homo Sapiens

<400> 220
Met Ser Thr Cys
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<210> 221
<211> 39
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<400> 221
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<210> 222
<211> 12
<212> PRT
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<400> 222
Met Ser Phe Phe Leu Val Ser Gln Arg Tyr Leu Arg
 1               5                   10

<210> 223
<211> 102
<212> DNA
<213> Homo Sapiens

<400> 223
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tatttctgtg aaactaacag gaagatattt tcagataact ag 102

<210> 224
<211> 33
<212> PRT
<213> Homo Sapiens

<400> 224
Met Arg Gln His Phe Leu Glu Ile Ile Thr Gln Val Ser Ser Met Leu
1 5 10 15
Asn Gly Thr Lys Tyr Phe Cys Glu Thr Asn Arg Lys Ile Phe Ser Asp
20 25 30
Asn

<210> 225
<211> 60
<212> DNA
<213> Homo Sapiens

<400> 225
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<210> 226
<211> 19
<212> PRT
<213> Homo Sapiens

<400> 226
Met Leu Asn Gly Thr Lys Tyr Phe Cys Glu Thr Asn Arg Lys Ile Phe
1 5 10 15
Ser Asp Asn

<210> 227
<211> 87
<212> DNA
<213> Homo Sapiens

<400> 227
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tgttgctttg ttacccagcc taattga 87

<210> 228
<211> 28
<212> PRT
<213> Homo Sapiens

<400> 228
Met Val Gln Asn Ile Ser Val Lys Leu Thr Gly Arg Tyr Phe Gln Ile
1 5 10 15
Thr Arg Ile Thr Cys Cys Phe Val Thr Gln Pro Asn
20 25

<210> 229
<211> 180
<212> DNA
<213> Homo Sapiens

<400> 229
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aaaattaaat ttagaataag aatgatttct ttaatttgtc ctttttttct ttggtctaaa 120
acattattaa atttttgtaa atattttgat ttaatgtgtc ttagatcctc attattttaa 180

<210> 230
<211> 59
<212> PRT
<213> Homo Sapiens

<400> 230
Met Leu Val Ser Lys Asn Gln Gln Lys Lys Lys Leu Arg Lys Leu Arg
1               5                   10                  15
Thr Asn Ile Lys Lys Ile Lys Phe Arg Ile Arg Met Ile Ser Leu Ile
            20                  25                  30
Cys Pro Phe Phe Leu Trp Ser Lys Thr Leu Leu Asn Phe Cys Lys Tyr
        35                  40                  45
Phe Asp Leu Met Cys Leu Arg Ser Ser Leu Phe
    50                  55

<210> 231
<211> 99
<212> DNA
<213> Homo Sapiens

<400> 231
atgatttctt taatttgtcc tttttttctt tggtctaaaa cattattaaa tttttgtaaa 60
tattttgatt taatgtgtct tagatcctca ttattttaa 99

<210> 232
<211> 32
<212> PRT
<213> Homo Sapiens

<400> 232
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1               5                   10                  15
Asn Phe Cys Lys Tyr Phe Asp Leu Met Cys Leu Arg Ser Ser Leu Phe
            20                  25                  30

<210> 233
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 233
atgtgtctta gatcctcatt attttaa 27

<210> 234
<211> 8
<212> PRT
<213> Homo Sapiens

<400> 234
Met Cys Leu Arg Ser Ser Leu Phe
1               5

<210> 235
<211> 150
<212> DNA
<213> Homo Sapiens

<400> 235
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gccaccaatt gtcatattat ttttagatga 150

<210> 236
<211> 49
<212> PRT

<213> Homo Sapiens

<400> 236
Met Leu Ile Cys Lys Val His Ala Ile Gln Ala Phe Lys Ser Asp Pro
1 5 10 15
His Pro Phe Ser Asn Met Tyr Leu Ser Ser His Tyr Phe Cys Phe Thr
20 25 30
Ala Val Leu Lys Asn Thr Tyr Tyr Ala Thr Asn Cys His Ile Ile Phe
35 40 45
Arg

<210> 237
<211> 54
<212> DNA
<213> Homo Sapiens

<400> 237
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<210> 238
<211> 17
<212> PRT
<213> Homo Sapiens

<400> 238
Met Pro Ser Arg His Leu Arg Ala Ile Leu Ile Pro Ser Ala Ile Cys
1 5 10 15
Ile

<210> 239
<211> 87
<212> DNA
<213> Homo Sapiens

<400> 239
atgtatttga gttcacacta tttctgtttt acagcagttt tgaaaaacac atactatgcc 60
accaattgtc atattatttt tagatga 87

<210> 240
<211> 28
<212> PRT
<213> Homo Sapiens

<400> 240
Met Tyr Leu Ser Ser His Tyr Phe Cys Phe Thr Ala Val Leu Lys Asn
1 5 10 15
Thr Tyr Tyr Ala Thr Asn Cys His Ile Ile Phe Arg
20 25

<210> 241
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 241
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<210> 242
<211> 13
<212> PRT
<213> Homo Sapiens

<400> 242

Met Pro Pro Ile Val Ile Leu Phe Leu Asp Asp Val Thr
1 5 10

<210> 243
<211> 15
<212> DNA
<213> Homo Sapiens

<400> 243
atgcctaata cttag 15

<210> 244
<211> 4
<212> PRT
<213> Homo Sapiens

<400> 244
Met Pro Asn Thr
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<210> 245
<211> 139
<212> DNA
<213> Homo Sapiens

<400> 245
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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 120
aaaaaaaaaa agggcggcc 139

<210> 246
<211> 46
<212> PRT
<213> Homo Sapiens

<400> 246
Met Ser Arg Asp His Phe Tyr Ile Lys Arg Glu Lys Lys Ser Lys Lys
1 5 10 15
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
20 25 30
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Gly Gly
35 40 45

<210> 247
<211> 1659
<212> DNA
<213> Homo Sapiens

<400> 247
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cctccagctc cttcctcctc ttcctcctcc tcctccacct ccggcttttg ggggatcact 120
gtcctctctc ggcagcagaa tgagccggca ggtggtccgc tccagcaagt tccgccacgt 180
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gcccacggtc tgtggccaca cagcccctgt gctagacatc gcctggtgcc cgcacaatga 420
caacgtcatt gccagtggct ccgaggactg cacagtcatg gtgtgggaga tcccagatgg 480
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gcacccagac acgatctaca gtgtggactg gagccgagat ggaggcctca tttgtacctc 720
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<210> 248
<211> 1386
<212> DNA
<213> Homo Sapiens

<400> 248
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gctgtcaacc ctaagtttgt ggccctgatc tgtgaggcca gcgggggagg ggccttcctg 180
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gtgggcactg gggcggccat gctgacactg ggcccagagg tgcacccaga cacgatctac 540
agtgtggact ggagccgaga tggaggcctc atttgtacct cctgccgtga caagcgcgtg 600
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acccggcccg tgcgtgcagt gttcgtgtcg gaggggaaga tcctgaccac gggcttcagc 720
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ctgcaggagc tggacaccag cagcggtgtc ctgctgccct tctttgaccc tgacaccaac 840
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gcccctttcc tgcactatct ctccatgttc agttccaagg agtcccagcg gggcatgggc 960
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gccagtggca ctcccagctc ggatgccgtg tctcggctgg aggaggagat gcggaagctc 1320
caggccacgg tgcaggagct ccagaagcgc ttggacaggc tggaggagac agtccaggcc 1380
aagtag 1386

<210> 249
<211> 461
<212> PRT
<213> Homo Sapiens

<400> 249
Met Ser Arg Gln Val Val Arg Ser Ser Lys Phe Arg His Val Phe Gly
1 5 10 15
Gln Pro Ala Lys Ala Asp Gln Cys Tyr Glu Asp Val Arg Val Ser Gln
20 25 30
Thr Thr Trp Asp Ser Gly Phe Cys Ala Val Asn Pro Lys Phe Val Ala
35 40 45
Leu Ile Cys Glu Ala Ser Gly Gly Gly Ala Phe Leu Val Leu Pro Leu
50 55 60
Gly Lys Thr Gly Arg Val Asp Lys Asn Ala Pro Thr Val Cys Gly His
65 70 75 80
Thr Ala Pro Val Leu Asp Ile Ala Trp Cys Pro His Asn Asp Asn Val
85 90 95
Ile Ala Ser Gly Ser Glu Asp Cys Thr Val Met Val Trp Glu Ile Pro
100 105 110
Asp Gly Gly Leu Met Leu Pro Leu Arg Glu Pro Val Val Thr Leu Glu

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        115                 120                 125
Gly His Thr Lys Arg Val Gly Ile Val Ala Trp His Thr Thr Ala Gln
    130                 135                 140
Asn Val Leu Leu Ser Ala Gly Cys Asp Asn Val Ile Met Val Trp Asp
145                 150                 155                 160
Val Gly Thr Gly Ala Ala Met Leu Thr Leu Gly Pro Glu Val His Pro
                165                 170                 175
Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys
            180                 185                 190
Thr Ser Cys Arg Asp Lys Arg Val Arg Ile Ile Glu Pro Arg Lys Gly
        195                 200                 205
Thr Val Val Ala Glu Lys Asp Arg Pro His Glu Gly Thr Arg Pro Val
    210                 215                 220
Arg Ala Val Phe Val Ser Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser
225                 230                 235                 240
Arg Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu
                245                 250                 255
Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu
            260                 265                 270
Pro Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly
        275                 280                 285
Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu
    290                 295                 300
His Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly
305                 310                 315                 320
Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg
                325                 330                 335
Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val
            340                 345                 350
Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala
        355                 360                 365
Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp
    370                 375                 380
Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys
385                 390                 395                 400
Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg
                405                 410                 415
Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg
            420                 425                 430
Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln
        435                 440                 445
Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
    450                 455                 460

<210> 250
<211> 75
<212> DNA
<213> Homo Sapiens

<400> 250
atgaagatgt gcgcgtctca cagaccacct gggacagtgg cttctgtgct gtcaacccta      60
agtttgtggc cctga                                                       75

<210> 251
<211> 24
<212> PRT
<213> Homo Sapiens

<400> 251
Met Lys Met Cys Ala Ser His Arg Pro Pro Gly Thr Val Ala Ser Val
 1               5                  10                  15
Leu Ser Thr Leu Ser Leu Trp Pro
            20

<210> 252
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<211> 69
<212> DNA
<213> Homo Sapiens

<400> 252
atgtgcgcgt ctcacagacc acctgggaca gtggcttctg tgctgtcaac cctaagtttg 60
tggccctga 69

<210> 253
<211> 22
<212> PRT
<213> Homo Sapiens

<400> 253
Met Cys Ala Ser His Arg Pro Pro Gly Thr Val Ala Ser Val Leu Ser
1 5 10 15
Thr Leu Ser Leu Trp Pro
20

<210> 254
<211> 39
<212> DNA
<213> Homo Sapiens

<400> 254
atgcgcccac ggtctgtggc cacacagccc ctgtgctag 39

<210> 255
<211> 12
<212> PRT
<213> Homo Sapiens

<400> 255
Met Arg Pro Arg Ser Val Ala Thr Gln Pro Leu Cys
1 5 10

<210> 256
<211> 72
<212> DNA
<213> Homo Sapiens

<400> 256
atgacaacgt cattgccagt ggctccgagg actgcacagt catggtgtgg gagatcccag 60
atgggggcct ga 72

<210> 257
<211> 23
<212> PRT
<213> Homo Sapiens

<400> 257
Met Thr Thr Ser Leu Pro Val Ala Pro Arg Thr Ala Gln Ser Trp Cys
1 5 10 15
Gly Arg Ser Gln Met Gly Ala
20

<210> 258
<211> 1068
<212> DNA
<213> Homo Sapiens

<400> 258
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ctgctcagtg caggttgtga caacgtgatc atggtgtggg acgtgggcac tggggcggcc 180

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atgctgacac tgggcccaga ggtgcaccca gacacgatct acagtgtgga ctggagccga      240
gatggaggcc tcatttgtac ctcctgccgt gacaagcgcg tgcgcatcat cgagccccgc      300
aaaggcactg tcgtagctga gaaggaccgt ccccacgagg ggacccggcc cgtgcgtgca      360
gtgttcgtgt cggaggggaa gatcctgacc acgggcttca gccgcatgag tgagcggcag      420
gtggcgctgt gggacacaaa gcacctggag gagccgctgt ccctgcagga gctggacacc      480
agcagcggtg tcctgctgcc cttctttgac cctgacacca acatcgtcta cctctgtggc      540
aagggtgaca gctcaatccg gtactttgag atcacttccg aggccccttt cctgcactat      600
ctctccatgt tcagttccaa ggagtcccag cggggcatgg gctacatgcc caaacgtggc      660
ctggaggtga acaagtgtga gatcgccagg ttctacaagc tgcacgagcg gaggtgtgag      720
cccattgcca tgacagtgcc tcgaaagtcg gacctgttcc aggaggacct gtacccaccc      780
accgcagggc ccgaccctgc cctcacggct gaggagtggc tgggggtcg ggatgctggg      840
cccctcctca tctccctcaa ggatggctac gtacccccaa agagccggga gctgagggtc      900
aaccggggcc tggacaccgg gcgcaggagg gcagcaccag aggccagtgg cactcccagc      960
tcggatgccg tgtctcggct ggaggaggag atgcggaagc tccaggccac ggtgcaggag     1020
ctccagaagc gcttggacag gctggaggag acagtccagg ccaagtag                  1068

<210> 259
<211> 355
<212> PRT
<213> Homo Sapiens

<400> 259
Met Val Trp Glu Ile Pro Asp Gly Gly Leu Met Leu Pro Leu Arg Glu
 1               5                  10                  15
Pro Val Val Thr Leu Glu Gly His Thr Lys Arg Val Gly Ile Val Ala
            20                  25                  30
Trp His Thr Thr Ala Gln Asn Val Leu Leu Ser Ala Gly Cys Asp Asn
        35                  40                  45
Val Ile Met Val Trp Asp Val Gly Thr Gly Ala Ala Met Leu Thr Leu
    50                  55                  60
Gly Pro Glu Val His Pro Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg
65                  70                  75                  80
Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys Arg Val Arg Ile
                85                  90                  95
Ile Glu Pro Arg Lys Gly Thr Val Val Ala Glu Lys Asp Arg Pro His
            100                 105                 110
Glu Gly Thr Arg Pro Val Arg Ala Val Phe Val Ser Glu Gly Lys Ile
        115                 120                 125
Leu Thr Thr Gly Phe Ser Arg Met Ser Glu Arg Gln Val Ala Leu Trp
    130                 135                 140
Asp Thr Lys His Leu Glu Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr
145                 150                 155                 160
Ser Ser Gly Val Leu Leu Pro Phe Phe Asp Pro Asp Thr Asn Ile Val
                165                 170                 175
Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr
            180                 185                 190
Ser Glu Ala Pro Phe Leu His Tyr Leu Ser Met Phe Ser Ser Lys Glu
        195                 200                 205
Ser Gln Arg Gly Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn
    210                 215                 220
Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu
225                 230                 235                 240
Pro Ile Ala Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp
                245                 250                 255
Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu
            260                 265                 270
Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp
        275                 280                 285
Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu
    290                 295                 300
Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser
305                 310                 315                 320
Ser Asp Ala Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala
                325                 330                 335
Thr Val Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val
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340 345 350
Gln Ala Lys
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<210> 260
<211> 12
<212> DNA
<213> Homo Sapiens

<400> 260
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<210> 261
<211> 3
<212> PRT
<213> Homo Sapiens

<400> 261
Met Gly Ala
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<210> 262
<211> 1038
<212> DNA
<213> Homo Sapiens

<400> 262
atgctgcccc tgcgggagcc cgtcgtcacc ctggagggcc acaccaagcg tgtgggcatt 60
gtggcctggc acaccacagc ccagaacgtg ctgctcagtg caggttgtga caacgtgatc 120
atggtgtggg acgtgggcac tggggcggcc atgctgacac tgggcccaga ggtgcaccca 180
gacacgatct acagtgtgga ctggagccga gatggaggcc tcatttgtac ctcctgccgt 240
gacaagcgcg tgcgcatcat cgagccccgc aaaggcactg tcgtagctga gaaggaccgt 300
ccccacgagg ggacccggcc cgtgcgtgca gtgttcgtgt cggaggggaa gatcctgacc 360
acgggcttca gccgcatgag tgagcggcag gtggcgctgt gggacacaaa gcacctggag 420
gagccgctgt ccctgcagga gctggacacc agcagcggtg tcctgctgcc cttctttgac 480
cctgacacca acatcgtcta cctctgtggc aagggtgaca gctcaatccg gtactttgag 540
atcacttccg aggccccttt cctgcactat ctctccatgt tcagttccaa ggagtcccag 600
cggggcatgg gctacatgcc caaacgtggc ctggaggtga acaagtgtga gatcgccagg 660
ttctacaagc tgcacgagcg gaggtgtgag cccattgcca tgacagtgcc tcgaaagtcg 720
gacctgttcc aggaggacct gtacccaccc accgcagggc ccgaccctgc cctcacggct 780
gaggagtggc tgggggtcg ggatgctggg cccctcctca tctccctcaa ggatggctac 840
gtacccccaa agagccggga gctgagggtc aaccggggcc tggacaccgg gcgcaggagg 900
gcagcaccag aggccagtgg cactcccagc tcggatgccg tgtctcggct ggaggaggag 960
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag 1020
acagtccagg ccaagtag 1038

<210> 263
<211> 345
<212> PRT
<213> Homo Sapiens

<400> 263
Met Leu Pro Leu Arg Glu Pro Val Val Thr Leu Glu Gly His Thr Lys
1 5 10 15
Arg Val Gly Ile Val Ala Trp His Thr Thr Ala Gln Asn Val Leu Leu
20 25 30
Ser Ala Gly Cys Asp Asn Val Ile Met Val Trp Asp Val Gly Thr Gly
35 40 45
Ala Ala Met Leu Thr Leu Gly Pro Glu Val His Pro Asp Thr Ile Tyr
50 55 60
Ser Val Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg
65 70 75 80
Asp Lys Arg Val Arg Ile Ile Glu Pro Arg Lys Gly Thr Val Val Ala
85 90 95
Glu Lys Asp Arg Pro His Glu Gly Thr Arg Pro Val Arg Ala Val Phe

100 105 110
Val Ser Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser Arg Met Ser Glu
115 120 125
Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu Pro Leu Ser
130 135 140
Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro Phe Phe Asp
145 150 155 160
Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile
165 170 175
Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His Tyr Leu Ser
180 185 190
Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys
195 200 205
Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu
210 215 220
His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser
225 230 235 240
Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro
245 250 255
Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu
260 265 270
Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu
275 280 285
Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu
290 295 300
Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu
305 310 315 320
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
325 330 335
Arg Leu Glu Glu Thr Val Gln Ala Lys
340 345

<210> 264
<211> 918
<212> DNA
<213> Homo Sapiens

<400> 264
atggtgtggg acgtgggcac tggggcggcc atgctgacac tgggcccaga ggtgcaccca 60
gacacgatct acagtgtgga ctggagccga gatggaggcc tcatttgtac ctcctgccgt 120
gacaagcgcg tgcgcatcat cgagccccgc aaaggcactg tcgtagctga gaaggaccgt 180
ccccacgagg ggacccggcc cgtgcgtgca gtgttcgtgt cggaggggaa gatcctgacc 240
acgggcttca gccgcatgag tgagcggcag gtggcgctgt gggacacaaa gcacctggag 300
gagccgctgt ccctgcagga gctggacacc agcagcggtg tcctgctgcc cttctttgac 360
cctgacacca acatcgtcta cctctgtggc aagggtgaca gctcaatccg gtactttgag 420
atcacttccg aggccccttt cctgcactat ctctccatgt tcagttccaa ggagtcccag 480
cggggcatgg gctacatgcc caaacgtggc ctggaggtga acaagtgtga gatcgccagg 540
ttctacaagc tgcacgagcg gaggtgtgag cccattgcca tgacagtgcc tcgaaagtcg 600
gacctgttcc aggaggacct gtacccaccc accgcagggc ccgaccctgc cctcacggct 660
gaggagtggc tgggggtcg ggatgctggg cccctcctca tctccctcaa ggatggctac 720
gtacccccaa agagccggga gctgagggtc aaccggggcc tggacaccgg gcgcaggagg 780
gcagcaccag aggccagtgg cactcccagc tcggatgccg tgtctcggct ggaggaggag 840
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag 900
acagtccagg ccaagtag 918

<210> 265
<211> 305
<212> PRT
<213> Homo Sapiens

<400> 265
Met Val Trp Asp Val Gly Thr Gly Ala Ala Met Leu Thr Leu Gly Pro
1 5 10 15
Glu Val His Pro Asp Thr Ile Tyr Ser Val Asp Trp Ser Arg Asp Gly
20 25 30

Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys Arg Val Arg Ile Ile Glu
35 40 45
Pro Arg Lys Gly Thr Val Val Ala Glu Lys Asp Arg Pro His Glu Gly
50 55 60
Thr Arg Pro Val Arg Ala Val Phe Val Ser Glu Gly Lys Ile Leu Thr
65 70 75 80
Thr Gly Phe Ser Arg Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr
85 90 95
Lys His Leu Glu Glu Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser
100 105 110
Gly Val Leu Leu Pro Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu
115 120 125
Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu
130 135 140
Ala Pro Phe Leu His Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln
145 150 155 160
Arg Gly Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys
165 170 175
Glu Ile Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile
180 185 190
Ala Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr
195 200 205
Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu
210 215 220
Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr
225 230 235 240
Val Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr
245 250 255
Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp
260 265 270
Ala Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val
275 280 285
Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala
290 295 300
Lys
305

<210> 266
<211> 888
<212> DNA
<213> Homo Sapiens

<400> 266
atgctgacac tgggcccaga ggtgcaccca gacacgatct acagtgtgga ctggagccga 60
gatggaggcc tcatttgtac ctcctgccgt gacaagcgcg tgcgcatcat cgagccccgc 120
aaaggcactg tcgtagctga gaaggaccgt ccccacgagg ggacccggcc cgtgcgtgca 180
gtgttcgtgt cggaggggaa gatcctgacc acgggcttca gccgcatgag tgagcggcag 240
gtggcgctgt gggacacaaa gcacctggag gagccgctgt ccctgcagga gctggacacc 300
agcagcggtg tcctgctgcc cttctttgac cctgacacca acatcgtcta cctctgtggc 360
aagggtgaca gctcaatccg gtactttgag atcacttccg aggccccttt cctgcactat 420
ctctccatgt tcagttccaa ggagtcccag cggggcatgg gctacatgcc caaacgtggc 480
ctggaggtga acaagtgtga gatcgccagg ttctacaagc tgcacgagcg gaggtgtgag 540
cccattgcca tgacagtgcc tcgaaagtcg gacctgttcc aggaggacct gtacccaccc 600
accgcagggc ccgaccctgc cctcacggct gaggagtggc tgggggggtcg ggatgctggg 660
cccctcctca tctccctcaa ggatggctac gtacccccaa agagccggga gctgagggtc 720
aaccggggcc tggacaccgg gcgcaggagg gcagcaccag aggccagtgg cactcccagc 780
tcggatgccg tgtctcggct ggaggaggag atgcggaagc tccaggccac ggtgcaggag 840
ctccagaagc gcttggacag gctggaggag acagtccagg ccaagtag 888

<210> 267
<211> 295
<212> PRT
<213> Homo Sapiens

<400> 267

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Met Leu Thr Leu Gly Pro Glu Val His Pro Asp Thr Ile Tyr Ser Val
 1               5                  10                  15
Asp Trp Ser Arg Asp Gly Gly Leu Ile Cys Thr Ser Cys Arg Asp Lys
            20                  25                  30
Arg Val Arg Ile Ile Glu Pro Arg Lys Gly Thr Val Val Ala Glu Lys
        35                  40                  45
Asp Arg Pro His Glu Gly Thr Arg Pro Val Arg Ala Val Phe Val Ser
    50                  55                  60
Glu Gly Lys Ile Leu Thr Thr Gly Phe Ser Arg Met Ser Glu Arg Gln
65                  70                  75                  80
Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu Pro Leu Ser Leu Gln
                85                  90                  95
Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro Phe Phe Asp Pro Asp
            100                 105                 110
Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp Ser Ser Ile Arg Tyr
        115                 120                 125
Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His Tyr Leu Ser Met Phe
    130                 135                 140
Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys Arg Gly
145                 150                 155                 160
Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu His Glu
                165                 170                 175
Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser Asp Leu
            180                 185                 190
Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro Ala Leu
        195                 200                 205
Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu Leu Ile
    210                 215                 220
Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu Arg Val
225                 230                 235                 240
Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu Ala Ser
                245                 250                 255
Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu Met Arg
            260                 265                 270
Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp Arg Leu
        275                 280                 285
Glu Glu Thr Val Gln Ala Lys
    290                 295
```

<210> 268
<211> 75
<212> DNA
<213> Homo Sapiens

<400> 268

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atggaggcct catttgtacc tcctgccgtg acaagcgcgt gcgcatcatc gagccccgca      60
aaggcactgt cgtag                                                       75
```

<210> 269
<211> 24
<212> PRT
<213> Homo Sapiens

<400> 269

```
Met Glu Ala Ser Phe Val Pro Pro Ala Val Thr Ser Ala Cys Ala Ser
 1               5                  10                  15
Ser Ser Pro Ala Lys Ala Leu Ser
            20
```

<210> 270
<211> 663
<212> DNA
<213> Homo Sapiens

<400> 270

atgagtgagc ggcaggtggc gctgtgggac acaaagcacc tggaggagcc gctgtccctg 60
caggagctgg acaccagcag cggtgtcctg ctgccсttct ttgaccctga caccaacatc 120
gtctacctct gtggcaaggg tgacagctca atccggtact ttgagatcac ttccgaggcc 180
cctttcctgc actatctctc catgttcagt tccaaggagt cccagcgggg catgggctac 240
atgcccaaac gtggcctgga ggtgaacaag tgtgagatcg ccaggttcta caagctgcac 300
gagcggaggt gtgagcccat tgccatgaca gtgcctcgaa agtcggacct gttccaggag 360
gacctgtacc cacccaccgc agggcccgac cctgccctca cggctgagga gtggctgggg 420
ggtcgggatg ctgggcccct cctcatctcc ctcaaggatg gctacgtacc cccaaagagc 480
cgggagctga gggtcaaccg ggcctggac accgggcgca ggagggcagc accagaggcc 540
agtggcactc ccagctcgga tgccgtgtct cggctggagg aggagatgcg gaagctccag 600
gccacggtgc aggagctcca gaagcgcttg gacaggctgg aggagacagt ccaggccaag 660
tag 663

<210> 271
<211> 220
<212> PRT
<213> Homo Sapiens

<400> 271
Met Ser Glu Arg Gln Val Ala Leu Trp Asp Thr Lys His Leu Glu Glu
1 5 10 15
Pro Leu Ser Leu Gln Glu Leu Asp Thr Ser Ser Gly Val Leu Leu Pro
20 25 30
Phe Phe Asp Pro Asp Thr Asn Ile Val Tyr Leu Cys Gly Lys Gly Asp
35 40 45
Ser Ser Ile Arg Tyr Phe Glu Ile Thr Ser Glu Ala Pro Phe Leu His
50 55 60
Tyr Leu Ser Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr
65 70 75 80
Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe
85 90 95
Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro
100 105 110
Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly
115 120 125
Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala
130 135 140
Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser
145 150 155 160
Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala
165 170 175
Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu
180 185 190
Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys
195 200 205
Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
210 215 220

<210> 272
<211> 462
<212> DNA
<213> Homo Sapiens

<400> 272
atgttcagtt ccaaggagtc ccagcggggc atgggctaca tgcccaaacg tggcctggag 60
gtgaacaagt gtgagatcgc caggttctac aagctgcacg agcggaggtg tgagcccatt 120
gccatgacag tgcctcgaaa gtcggacctg ttccaggagg acctgtaccc acccaccgca 180
gggcccgacc ctgccctcac ggctgaggag tggctggggg gtcgggatgc tgggcccctc 240
ctcatctccc tcaaggatgg ctacgtaccc ccaaagagcc gggagctgag ggtcaaccgg 300
ggcctggaca ccgggcgcag gagggcagca ccagaggcca gtggcactcc cagctcggat 360
gccgtgtctc ggctggagga ggagatgcgg aagctccagg ccacggtgca ggagctccag 420
aagcgcttgg acaggctgga ggagacagtc caggccaagt ag 462

<210> 273
<211> 153

<212> PRT
<213> Homo Sapiens

<400> 273
Met Phe Ser Ser Lys Glu Ser Gln Arg Gly Met Gly Tyr Met Pro Lys
1 5 10 15
Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe Tyr Lys Leu
20 25 30
His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro Arg Lys Ser
35 40 45
Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly Pro Asp Pro
50 55 60
Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala Gly Pro Leu
65 70 75 80
Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser Arg Glu Leu
85 90 95
Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala Ala Pro Glu
100 105 110
Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu Glu Glu Glu
115 120 125
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
130 135 140
Arg Leu Glu Glu Thr Val Gln Ala Lys
145 150

<210> 274
<211> 432
<212> DNA
<213> Homo Sapiens

<400> 274
atgggctaca tgcccaaacg tggcctggag gtgaacaagt gtgagatcgc caggttctac 60
aagctgcacg agcggaggtg tgagcccatt gccatgacag tgcctcgaaa gtcggacctg 120
ttccaggagg acctgtaccc acccaccgca gggcccgacc ctgccctcac ggctgaggag 180
tggctggggg gtcgggatgc tgggcccctc ctcatctccc tcaaggatgg ctacgtaccc 240
ccaaagagcc gggagctgag ggtcaaccgg ggcctggaca ccgggcgcag gagggcagca 300
ccagaggcca gtggcactcc cagctcggat gccgtgtctc ggctggagga ggagatgcgg 360
aagctccagg ccacggtgca ggagctccag aagcgcttgg acaggctgga ggagacagtc 420
caggccaagt ag 432

<210> 275
<211> 143
<212> PRT
<213> Homo Sapiens

<400> 275
Met Gly Tyr Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile
1 5 10 15
Ala Arg Phe Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met
20 25 30
Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro
35 40 45
Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly
50 55 60
Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro
65 70 75 80
Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg
85 90 95
Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val
100 105 110
Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu
115 120 125
Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
130 135 140

<210> 276
<211> 423
<212> DNA
<213> Homo Sapiens

<400> 276
atgcccaaac gtggcctgga ggtgaacaag tgtgagatcg ccaggttcta caagctgcac 60
gagcggaggt gtgagcccat tgccatgaca gtgcctcgaa agtcggacct gttccaggag 120
gacctgtacc cacccaccgc agggcccgac cctgccctca cggctgagga gtggctgggg 180
ggtcgggatg ctgggcccct cctcatctcc ctcaaggatg gctacgtacc cccaaagagc 240
cgggagctga gggtcaaccg gggcctggac accgggcgca ggagggcagc accagaggcc 300
agtggcactc ccagctcgga tgccgtgtct cggctggagg aggagatgcg gaagctccag 360
gccacggtgc aggagctcca gaagcgcttg gacaggctgg aggagacagt ccaggccaag 420
tag 423

<210> 277
<211> 140
<212> PRT
<213> Homo Sapiens

<400> 277
Met Pro Lys Arg Gly Leu Glu Val Asn Lys Cys Glu Ile Ala Arg Phe
1 5 10 15
Tyr Lys Leu His Glu Arg Arg Cys Glu Pro Ile Ala Met Thr Val Pro
20 25 30
Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro Pro Thr Ala Gly
35 40 45
Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly Gly Arg Asp Ala
50 55 60
Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val Pro Pro Lys Ser
65 70 75 80
Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly Arg Arg Arg Ala
85 90 95
Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala Val Ser Arg Leu
100 105 110
Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys
115 120 125
Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
130 135 140

<210> 278
<211> 339
<212> DNA
<213> Homo Sapiens

<400> 278
atgacagtgc ctcgaaagtc ggacctgttc caggaggacc tgtacccacc caccgcaggg 60
cccgaccctg ccctcacggc tgaggagtgg ctggggggtc gggatgctgg gcccctcctc 120
atctccctca aggatggcta cgtaccccca aagagccggg agctgagggt caaccggggc 180
ctggacaccg ggcgcaggag ggcagcacca gaggccagtg gcactcccag ctcggatgcc 240
gtgtctcggc tggaggagga gatgcggaag ctccaggcca cggtgcagga gctccagaag 300
cgcttggaca ggctggagga gacagtccag gccaagtag 339

<210> 279
<211> 112
<212> PRT
<213> Homo Sapiens

<400> 279
Met Thr Val Pro Arg Lys Ser Asp Leu Phe Gln Glu Asp Leu Tyr Pro
1 5 10 15
Pro Thr Ala Gly Pro Asp Pro Ala Leu Thr Ala Glu Glu Trp Leu Gly
20 25 30
Gly Arg Asp Ala Gly Pro Leu Leu Ile Ser Leu Lys Asp Gly Tyr Val
35 40 45

Pro Pro Lys Ser Arg Glu Leu Arg Val Asn Arg Gly Leu Asp Thr Gly
    50                  55                  60
Arg Arg Arg Ala Ala Pro Glu Ala Ser Gly Thr Pro Ser Ser Asp Ala
65                  70                  75                  80
Val Ser Arg Leu Glu Glu Glu Met Arg Lys Leu Gln Ala Thr Val Gln
                85                  90                  95
Glu Leu Gln Lys Arg Leu Asp Arg Leu Glu Glu Thr Val Gln Ala Lys
            100                 105                 110

<210> 280
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 280
atgctgggcc cctcctcatc tccctcaagg atggctacgt acccccaaag agccgggagc 60
tga 63

<210> 281
<211> 20
<212> PRT
<213> Homo Sapiens

<400> 281
Met Leu Gly Pro Ser Ser Ser Pro Ser Arg Met Ala Thr Tyr Pro Gln
1               5                   10                  15
Arg Ala Gly Ser
            20

<210> 282
<211> 33
<212> DNA
<213> Homo Sapiens

<400> 282
atggctacgt acccccaaag agccgggagc tga 33

<210> 283
<211> 10
<212> PRT
<213> Homo Sapiens

<400> 283
Met Ala Thr Tyr Pro Gln Arg Ala Gly Ser
1               5                   10

<210> 284
<211> 238
<212> DNA
<213> Homo Sapiens

<400> 284
atgccgtgtc tcggctggag gaggagatgc ggaagctcca ggccacggtg caggagctcc 60
agaagcgctt ggacaggctg gaggagacag tccaggccaa gtagagcccc gcagggcctc 120
cagcagggtc agccattcac acccatccac tcacctccca ttcccagcca catggcagag 180
aaaaaaatca taataaaatg gctttatttt ctggtaaaaa aaaaaaaaaa gggcggcc 238

<210> 285
<211> 79
<212> PRT
<213> Homo Sapiens

<400> 285
Met Pro Cys Leu Gly Trp Arg Arg Arg Cys Gly Ser Ser Arg Pro Arg
1               5                   10                  15

```
Cys Arg Ser Ser Arg Ser Ala Trp Thr Gly Trp Arg Arg Gln Ser Arg
            20                  25                  30
Pro Ser Arg Ala Pro Gln Gly Leu Gln Gln Gly Gln Pro Phe Thr Pro
        35                  40                  45
Ile His Ser Pro Pro Ile Pro Ser His Met Ala Glu Lys Lys Ile Ile
    50                  55                  60
Ile Lys Trp Leu Tyr Phe Leu Val Lys Lys Lys Lys Lys Gly Gly
65                  70                  75

<210> 286
<211> 78
<212> DNA
<213> Homo Sapiens

<400> 286
atgcggaagc tccaggccac ggtgcaggag ctccagaagc gcttggacag gctggaggag      60
acagtccagg ccaagtag                                                    78

<210> 287
<211> 25
<212> PRT
<213> Homo Sapiens

<400> 287
Met Arg Lys Leu Gln Ala Thr Val Gln Glu Leu Gln Lys Arg Leu Asp
 1               5                  10                  15
Arg Leu Glu Glu Thr Val Gln Ala Lys
            20                  25

<210> 288
<211> 67
<212> DNA
<213> Homo Sapiens

<400> 288
atggcagaga aaaaaatcat aataaaatgg ctttattttc tggtaaaaaa aaaaaaaaag      60
ggcggcc                                                                67

<210> 289
<211> 22
<212> PRT
<213> Homo Sapiens

<400> 289
Met Ala Glu Lys Lys Ile Ile Ile Lys Trp Leu Tyr Phe Leu Val Lys
 1               5                  10                  15
Lys Lys Lys Lys Gly Gly
            20

<210> 290
<211> 41
<212> DNA
<213> Homo Sapiens

<400> 290
atggctttat tttctggtaa aaaaaaaaaa aaagggcggc c                         41

<210> 291
<211> 13
<212> PRT
<213> Homo Sapiens

<400> 291
Met Ala Leu Phe Ser Gly Lys Lys Lys Lys Lys Gly Arg
 1               5                  10
```

<210> 292
<211> 722
<212> DNA
<213> Homo Sapiens

<400> 292
tcgacccacg cgtccgctct gccgcagcct gtgccgccgc cgcctcctgg gaagagagga 60
agcgggagag gagcccacgt ctcctgtcac ccaatatctc cagccgcgca gtcccgaaga 120
gtgtaagatg ttcgcctgcg ccaagctcgc ctgcaccccc tctctgatcc gagctggatc 180
cagagttgca tacagaccaa tttctgcatc agtgttatct cgaccagagg ctagtaggac 240
tggagagggc tctacggtat ttaatggggc ccagaatggt gtgtctcagc taatccaaag 300
ggagtttcag accagtgcaa tcagcagaga cattgatact gctgccaaat ttattggtgc 360
aggtgctgca acagtaggag tggctggttc tggtgctggt attggaacag tctttggcag 420
ccttatcatt ggttatgcca gaaacccttc gctgaagcag cagctgttct catatgctat 480
cctgggattt gccttgtctg aagctatggg tctcttttgt ttgatggttg ctttcttgat 540
tttgtttgcc atgtaacaaa ttactgcttg acatgttggc attcatatta attacggatg 600
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cagaccagtg caatcagcag agacattgat actgctgcca aatttattgg tgcaggtgct 240
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Pro Glu Ala Ser Arg Thr Gly Glu Gly Ser Thr Val Phe Asn Gly Ala
35 40 45
Gln Asn Gly Val Ser Gln Leu Ile Gln Arg Glu Phe Gln Thr Ser Ala
50 55 60
Ile Ser Arg Asp Ile Asp Thr Ala Ala Lys Phe Ile Gly Ala Gly Ala
65 70 75 80
Ala Thr Val Gly Val Ala Gly Ser Gly Ala Gly Ile Gly Thr Val Phe
85 90 95
Gly Ser Leu Ile Ile Gly Tyr Ala Arg Asn Pro Ser Leu Lys Gln Gln
100 105 110
Leu Phe Ser Tyr Ala Ile Leu Gly Phe Ala Leu Ser Glu Ala Met Gly
115 120 125
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<210> 296
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1 5

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1 5 10 15

<210> 303
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<211> 10
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1 5 10

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<211> 51
<212> DNA
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1 5 10 15

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<212> DNA
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aaaaaagggc ggcc 74

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cttcacaaac ccagtgtttt aggagcatga gtgccgtgtg tgtgcgtcct gtcggagccc     300
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ggcc                                                                  364

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<210> 314
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<212> DNA
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<210> 316
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<212> DNA
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<210> 317
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<211> 1046
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ctaaaactca cctgagttgt aaaaaactcc agttgacaca aaatagacta cgaaagtggc 360
tttaacatat ctgaacacac aatagctaag acccaaactg ggattagata ccccactatg 420
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cagcttaaaa ctcaaaggac ctggcggtgc ttcatacccc tctagaggag cctgttctgt 540
aatcgataaa ccccgatcaa cctcaccacc tcttgctcag cctatatacc gccatcttca 600
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aaaaaaaaaa aaaaaaaagg gcggcc 1046

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<212> DNA
<213> Homo Sapiens

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<210> 328
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tag 63

<210> 330
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Val Ile Asn Leu
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<210> 331
<211> 108
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ccacagctta aaactcaaag gacctggcgg tgcttcatac ccctctag 108

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<400> 332
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1 5 10 15
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20 25 30
Ile Pro Leu
35

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<210> 334
<211> 5
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<210> 335
<211> 51
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1 5 10 15

<210> 337
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<210> 338
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<210> 341
<211> 152
<212> DNA
<213> Homo Sapiens

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aaaaaaaaaa aaaaaaaaaa aaaagggcgg cc 152

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<211> 50
<212> PRT
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1 5 10 15
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
20 25 30
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
35 40 45
Gly Arg
50

International Application No: PCT/

## MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on pages 116-117, lines 18-31 and 1-12 of the description

**A. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

**American Type Culture Collection**

Address of depositary institution (including postal code and country)

10801 University Blvd.
Manassas, VA 20110-2209
US

Date of deposit March 16, 2000 Accession Number PTA-1492

**B. ADDITIONAL INDICATIONS** (leave blank if not applicable). This information is continued on a separate attached sheet

**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not all designated States)

**D. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the International application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

# PATENT COOPERATION TREATY

# PCT

## DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter* and 39)

| Applicant's or agent's file reference 10001-006-228 | IMPORTANT DECLARATION | Date of mailing *(day/month/year)* 17 JUL 2001 |
|---|---|---|
| International application No. PCT/US01/11655 | International filing date *(day/month/year)* 09 APRIL 2001 | (Earliest) Priority Date *(day/month/year)* 11 APRIL 2000 |
| International Patent Classification (IPC) or both national classification and IPC Please See Continuation Sheet. | | |
| Applicant COGENT NEUROSCIENCE, INC. | | |

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories.
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☐ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practiced on the human or animal body.
   - l. ☐ mere presentations of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.

2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   ☒ the description ☒ the claims ☒ the drawings

3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out.

   ☒ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

4. Further comments:
   Please See Continuation Sheet.

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | MARY TUNG |
| Form PCT/ISA/203 (July 1998)* | (703) 308-0196 |

**DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US01/11655

The International Patent Classification (IPC) or National Classification and IPC are as listed below:

IPC(7): A61K 31/7088, 38/00, 39/395; C12N 1/20, 15/12; C12P 19/34, 21/06; G01N 33/53 US Cl.: 424/139.1; 435/7.1, 69.1, 91.1, 252.3, 320.1; 514/12, 44; 536/23.5

4. Further Comments (Continued):

A meaningful search cannot be performed on the instant claims.
The claims appear to encompass an unfathomable number of inventions because of the number of claimed sequences.

It is noted that the instant claim set is apparently drawn to a multitude of DNA or amino acid sequences shown in Figures 4-13. Each of Figures 4-13 are apparently broken up into a multitude of subfigures. Claim 1 (a) apparently refers to "an amino acid sequence which is shown in all of Figures 4A-AB". It is not at all clear how one sequence can be the same as all those separate, distinct sequences. Perhaps applicant intended to refer to the various subfigures in the alternative? Or maybe the open claim language "comprising" as in am amino acid sequence which comprises all of those shown in Figures 4A-AB.

Figures 4-13 are defective because the sheets are not numbered in consecutive Arabic numbers. See PCT/RO/106 mailed May 4, 2001. Further, the first sheet of each of the Figures 4-13 contains a heading "open reading frame for..." and the text "Fig No." These words are missing from the subsequent sheets of each of the subfigures 4-13. The figures are not clearly labeled. Further, wtih regard to the headings, PCT Rule 11.11(a) prohibits words in the drawings.

Each figure does not have a uniques label which says "Fig. No. 4A, Figure No. 4B", etc. Some of the figures apparently contain subfigures which run over onto the next sheet. See 10L, 10J,etc.Further, some of the figures apprently contain subfigures which so not have a figure label, see the text in the box above Figure 10P. Furthermore, the numbering system of the sequences appears to be incorrect, see numbers 340 and 341, both denoting the same position of the last line of amino acids of Figure 10H. None of the abberations are permissible and a search of any such material would not be meaningful.

Beyond all these errors, the overriding problem with performing a search on the claims is that in the figures and of the Brief Description of the Figures, pages 13-15, no SEQ ID Nos are provided. Without any such correlation, it is impossible to determine which of te sequences recited int he claims correspond to those recited in the sequence listing. Absent that correlation, it is impossible to determine the full scope of the claimed invention or to search even a portion of the claimed invention.

PCT Rule 6.2(a) states that the claims shall not, except when absolutely necessary, rely, in respect to the technical features of the invention, on references to the description of the drawings. In particular, they shall not rely on such references as "as described in part...of the description" or "as illustrated in figure...of the drawings". The nature of the instant invention does not meet the criteria of "absolutely necessary" because these claims could have easily been drafted to include the particular SEQ ID Nos of the sequences. That may result in theclaim becoming rather lengthy, but this is to be expected when one claim appears to encompass hundreds of inventions.

The Authorized Officer contacted the applicants on 16 May 2001 concerning a potential Lack of Unity. However, upon further consideration, the claims have been determined to be unsearchable.